1942

Life cycle of Capillaria caudinflata, a nematode parasite of the common fowl

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NOTE TO USERS

LIFE CYCLE OF CAPILLARIA CAUDINFLATA, A NEMATODE PARASITE OF THE COMMON FOWL

by

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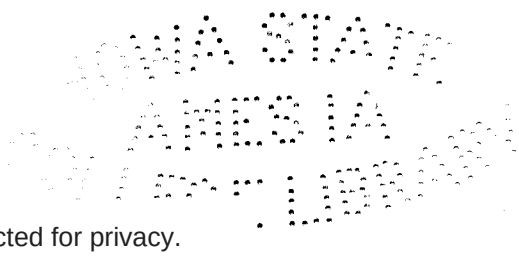
A Thesis Submitted to the Graduate Faculty
for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject: Zoology



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INTRODUCTION

Post-mortem examination of the digestive tracts of several specimens of the common fowl, Gallus domesticus, received at Charles City, Iowa, revealed the presence of numerous nematodes of the genus Capillaria. A few of the worms, sent to the Bureau of Animal Industry, Department of Agriculture, Washington, D. C., in May, 1938 were identified by Dr. E. E. Wehr as Capillaria caudinflata. On subsequent examination of other chickens received from various parts of the country, it was observed that infestation with capillaria worms was commonly associated with severe enteritis, diarrhea and emaciation. Since these worms appeared to be of considerable economic importance, an investigation of capillaria worms in chickens was desirable. Preliminary geographical distribution studies indicated that Capillaria caudinflata was by far the most prevalent species in the middle west and that an investigation of capillariasis in this region would be chiefly an investigation of Capillaria caudinflata.

A survey of the literature concerning this capillarid showed that nothing had been reported on its occurrence in the United States; that much of the existing literature reported in other countries was inaccurate or confusing;

and that nothing was known of the method whereby the parasite is transmitted from one fowl to another or of the environmental factors which might influence such transmission. Furthermore, inquiry sent to the Chief of the Zoological Division of the Bureau of Animal Industry revealed that research workers of that division had done no work on the life-history of C. caudinflata. Thus the life-history of this nematode appeared to be an important helminthological problem remaining untouched by other parasitologists. Early in November, 1938, the writer began an investigation of the life-history together with other related factors.

Having obtained considerable evidence of the transmission of C. caudinflata by earthworms of the species Helodrilus caliginosus, the writer sent specimens of these earthworms to the Zoological Division, Bureau of Animal Industry, on March 27, 1942 requesting confirmation of the earthworm species and confiding the information that data had been obtained showing that earthworms in the vicinity of Charles City, Iowa serve as intermediate hosts for one of the important parasites of poultry. Receipt of these earthworms was promptly acknowledged by Dr. Price and later by a letter from Dr. Wehr.

Just as the discussion and conclusions of this paper were being written, preparatory to its submission to the graduate faculty as a doctoral thesis, a note by R. W. Allen and E. E. Wehr (1942) entitled, "Earthworms as possible intermediate

hosts of Capillaria caudinflata of the chicken and turkey" appeared in the Proceedings of the Helminthological Society of Washington. These authors apparently showed that C. caudinflata may be transmitted through earthworms to chickens and turkeys, although their experiments were not controlled and therefore failed to prove that the earthworms served as true intermediate hosts.

According to the note by Allen and Wehr, all results of their experiments were obtained subsequent to March 27, 1942. It appears that these investigators had no evidence of transmission until April 18, 1942, whereas experiments reported in this paper show that the writer had obtained evidence of transmission to chickens some 5 months before these investigators began their experiments. It should be further noted that the writer had transmitted C. caudinflata to turkeys about 2 weeks earlier than the date recorded by Allen and Wehr.

Prior to 1936, no capillarids were known to require intermediate hosts. Capillaria columbae (Rudolphi, 1819), probably the most widely recognized species found in chickens, was known to have a direct life-cycle. This is also true of Capillaria contorta (Creplin, 1839), a parasite of the upper digestive tract of many species of birds and of Capillaria hepatica (Hall, 1916), a parasite of rats, rabbits and man. Furthermore, a direct life-cycle was known for the human whipworm, Trichuris trichiura (Linnaeus, 1771), a parasite closely

related to the capillarids. Consequently it was generally supposed that the various capillarid species have direct life-cycles. However, Wehr (1936) showed that the crop-worm of chickens, Capillaria annulata (Molin, 1858), may be transmitted by certain species of earthworms, thus suggesting the possibility that other capillarids may also require intermediate hosts.

A. Purpose of the Study

It is the purpose of this study to review the work of previous investigators who have dealt with Capillaria caudinflata (Molin, 1858); to study its geographical distribution in the United States; and to determine its life-cycle. Considerable emphasis is placed on the reaction of the ova, during their exogenous development, to environmental conditions, since a better knowledge of these factors may lead to practical methods of control.

REVIEW OF LITERATURE

Literature concerning nematodes which are now placed in the genus Capillaria has appeared from time to time for more than a century and a quarter. It has been said that in no other group of roundworms is our knowledge so incomplete. Until recently the majority of investigators have dealt chiefly with the morphology and systematic position of these worms with little consideration given to such problems as life-cycles, geographical distribution, host-specificity or pathogenicity. As a matter of fact, there has been much disagreement among various authors concerning the classification of several species. Such disagreement would seem inevitable in view of the brief and in many cases inaccurate descriptions by early observers.

Although much space has been given in the literature to discussion of the classification of capillarid worms, it is seldom that two writers have agreed to all the details of a given classification. All writers, however, seem to have agreed that the phylum is Nemathelminthes and the class is Nematoda, but at this point the divergence of opinion begins.

Ward (1917) considered that there was a basic difference in the structure of the esophagus of parasitic nematodes whereby he could separate them into two groups. Accordingly

he divided them into two sub-orders, the Myosyringata whose members were characterized by a prominent muscular esophagus having a typical tri-radiate lumen, and the Trichosyringata with esophagus slender, non-muscular and its lumen a capillary chitinous tube traversing a row of granular cells. In 1927, Cram considered Ward's two sub-orders as orders and in 1936 Neveu-Lemaire further promoted them to the rank of sub-class. However, Chandler (1936) cited the work of Chitwood (1930) demonstrating the absence of any fundamental peculiarity in the esophagus of the trichuroid worms and on this basis declined to recognize such a division of the Nematoda.

There are at least 5 different names proposed by various authors for the order to which the Capillaria belong. Yorke and Maplestone (1926) classified the typical nematodes as order Eunematoda Ward, 1916, to dinstinguish them from a second order Gordiacea, Siebold, 1848 (quoted by Carus, 1863). The super-family name, Trichinelloidea, proposed by Hall (1916) has been raised to the rank of order by Baylis and Daubney (1926), and by Hegner, Root and Augustine (1929) although Cameron (1934), an English author, still used Trichinelloidea to designate the super-family. Cram (1927) as previously mentioned, used the term Trichosyringata for this order while Chitwood (1930) used an entirely different term, Trichuroidea. A fifth term used by Chandler (1936) and Neveu-Lemaire (1936) was Trichurata.

Few attempts have been made to sub-divide the order to which the genus Capillaria belongs. Molin (1861) placed the capillarids in the sub-order Proctucha, and Ward (1918) called them Trichosyringata while Neveu-Lemaire used the name Trichuroidea Railliet, 1916.

The only super-family name which has been found is that proposed by Hall (1916) as mentioned above. Diesing (1861) gave the name Trichotrachelidae to the family of which the genus Capillaria is a member. On this point of classification he has been followed by Eberth (1863) and Shipley (1909). However, the more commonly accepted name is Trichinellidae Stiles and Crane, 1910, although Travassos (1915) chose to restrict this family to the single genus Trichinella. Since Travassos did not regard Diesing's name Trichotrachelidae, as following the rules of zoological nomenclature, he proposed the name Trichuridae to include the following genera: Trichuris Roederer and Wagler, 1761--type genus; Trichosomoides Railliet, 1895; Capillaria Zeder, 1800; Sclerotrichum Rudolphi, 1819; and Onchophora Diesing, 1851. He considered that the first three genera constitute a natural group, much removed from the genus Trichinella and included the genera Sclerotrichum and Onchophora merely because each was erected for a single species, stating that the meager knowledge we have of them does not permit a valid opinion on their systematic position. Numerous authors including Hall (1916), Ward (1918), Irwin-Smith

(1920), Baylis and Daubney (1926), and Hegner, Root and Augustine (1929), have not followed Travassos' suggestion since they used the name Trichinellidae for this family. In 1936, Neveu-Lemaire proposed "Capillaridae fam. nov." for the single genus Capillaria Zeder, 1800. He described the family as, "Trichuroidea in which the esophageal portion of the body is shorter than the posterior portion or rarely equal to it, the posterior part of the body being a little larger than the anterior".[1]

Only 2 sub-family names have been found in the literature, the name Trichurinae Ransom, 1911 and Capillariinae Railliet, 1915. The name porposed by Ransom has been accepted by Hall (1916), Ward (1918), Irwin-Smith (1920), Baylis and Daubney (1926) and Hegner, Root and Augustine (1929) while Yorke and Maplestone (1926) have adopted the name suggested by Railliet.

Zeder (1800) appears to have been the first to propose a generic name for the group of worms now placed in the genus Capillaria. A few years later, Rudolphi (1819) described another genus which he called Trichosoma and listed as "Genus II. Trichosoma (Capillaria, Zederi)"[2] thus indicating that he recognized the synonymy of the two terms.

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[1]Neveu-Lemaire, M. Traité d'helminthologie médicale et veterinaire. Paris. 1936. P. 1305. (Translation by N.F.M.).

[2]Rudolphi, C. A. Entozoorum Synopsis cui accedunt mantissa duplex et indices locupletissimi. Berolini. 1819. p. 13.

Consequently there can be no question of the priority of Zeder's genus, Capillaria. Since Creplin (1839) used the term Trichosomum to rename Trichosoma Rud., his generic name must also be considered synonymous with Capillaria Zeder, 1800.

Dujardin (1845) proposed the division of the genus Trichosoma Rudolphi, 1819 into 5 genera, Trichosoma, Thominx, Calodium, Liniscus and Eucoleus. However, Baylis (1931), who gave an excellent discussion of Dujardin's work together with a translation of the key for the identification of his genera, pointed out that such characters as the length of spicule sheath, the presence or absence of a spicule and the presence of spines on the spicule sheath are too variable to make them of generic significance. Baylis also pointed out that the genus Hepaticola Hall, 1916 described as having no bacillary bands and no spicule is not valid because he found both characters present in specimens which he has studied. On this basis Baylis treated Hepaticola Hall, 1916 as a synonym of Capillaria Zeder, 1800 and redefined the genus Capillaria as follows:

Capillaria Zeder, 1800[1]

Synonyms: Trichosoma Rudolphi, 1819; Trichosomum Creplin, 1839; Calodium Dujardin, 1845; Thominx Dujardin, 1845; Liniscus Dujardin, 1845; Eucoleus Dujardin, 1845; Hepaticola Hall, 1916.

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[1]Baylis, H. A. On the structure and relationships of the nematode, Capillaria (Hepaticola) hepatica (Bancroft) Parasitology 23 (4):541. 1931.

Trichinellidae, Trichurinae: Body very slender. Oesophageal portion usually shorter than the posterior portion, which is only slightly thicker. One or more longitudinal (dorsal, ventral or lateral) 'bacillary bands' usually present. Male with a protrusible, membranous copulatory sheath, or spicule-sheath, the lining of which (the outer surface when everted) may be smooth or spiny. A spicule is usually present, but may be very slightly chitinized or absent. The caudal end of the male is frequently provided with delicate alae or a bursa-like structure. Vulva of female close behind the junction of the oesophagus and intestine, and often provided with a protrusible, membranous, funnel-like structure. Eggs barrel-shaped or lemon-shaped, with polar opercula. The surface of the outer layer of the egg-shell may be smooth or variously ornamented.

Hab.: Alimentary canal, liver, urinary bladder or respiratory passages of all groups of vertebrates.

Genotype: C. (Trichocephalus) anatis (Schrank, 1790) (=C. tumida Zeder, 1803 = Trichocephalus capillaris Rudolphi, 1809 = Trichosoma brevicolle Rudolphi, 1819).

In the year 1858, Molin described a new species of nematode from Perdix coturnix which he called Calodium caudinflatum. The species was described as follows:

Calodium caudinflatum Molin[1]

Corpus capillare, maris utrinque, feminae retrorsum attenuatum; extremitas caudalis maris epidermide in bullam magnam ellipsoidicam, transparentem inflata; vagina penis tubulosa, transversim striata, penisque filiformis longissimi e bursa terminali, in apice caudali sursum excisa mucroni brevi opposita extantes; extremitas caudalis feminae apice rotundato; hiatus ani subterminalis, lateralis; apertura vulvae bursa prominula in anteriori corporis parte, hiatu bilabiato, labio interno longiori. Longit mar. 0.017; fem. 0.025.

Habitaculum. Perdix Coturnix: in intestino tenui, Junio, Patavii (Molin).

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[1] Molin, Rafael, (1858) Prospectus helminthum, quae in parte secunda prodromi faunae helminthilogica Venetae continentur. Sitzungs. K. Akad. Wissensch. Wien. Math.-Naturw. Cl. 1858. 33:302.

A translation of Molin's article follows:[1]

Body capillary, the male on both sides (probably means throughout the length of the body), the female tapering anteriorly; the epidermis of the caudal extremity of the male inflated into a large transparent elypsoidal bubble; the spicule sheath tubular, transversely striated, spicule very long and thread-like extending from the terminal bursa opposite a point cut in, from below, in the caudal end; caudal extremity of female (with) rounded-off end; opening of anus subterminal, lateral; opening of vulval bursa projecting out in the anterior part of the body, opening bilobed, inner lip the longest. Length of male 17 mm.; female 25 mm.

Habitat: Perdix coturnix: in small intestine, Junio Patavii (Molin).

In 1861 Molin again published, with one exception, the same description together with figures of the caudal portion of the male and the region of the genital aperture of the female. In his first paper he described the inner lip of the vulval bursa as the longest ("labio interno longiori") but in 1861 he described it as "labio externo longiori". Since his figures of this region of the female shows the outer lip the longest his earlier description was evidently erroneous. Molin's description together with his figures leave little doubt that he was describing the same species which the writer has found in chickens of the United States.

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[1]Translation by N.F.M.

Rudolphi (1819) in his Entozoorum Synopsis described a new genus which he called Trichosoma. Under this genus, he described the species Trichosoma longicolle R. as occurring in certain galliform birds. The writer has found little information in his description, however, to indicate just what species of "Trichosoma" he may have examined. Probably the most valuable clue given is the hosts from which his worms were obtained, that is, "Hab. In intestinis praesertim crassis Phasiani galli et colchici; in coecis Tetraonis Urogalli, Perdicis et Tetricis."[1] The apparent lack of host-specificity among capillarids as a group, and the fact that several species may occur in the digestive tract of a single host at any one time even makes this information of little value to the taxonomist. The morphological characteristics given by Rudolphi offer no clue by which his worms could possibly be distinguished from other closely related species. As a matter of fact, Shipley (1909) suggested that Rudolphi may have been dealing with more than one species of "trichosomes" which seems quite likely in view of the fact that part of them were obtained from the intestine and part of them from the cecum of the various hosts.

Trichosoma longicolle R. was re-described by Dujardin (1845) who listed earlier ill-defined synonyms; also by Eberth (1863) who gave a short description and a figure of a nematode

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[1]Rudolphi, op. cit., p. 14.

from the cecum of the chicken. However, as Railliet (1895) pointed out, Eberth's identification was contradictory to that of Rudolphi and he believed that Eberth was dealing with T. retusum Railliet, 1893. Railliet also believed that the "trichosomes" which Dujardin called T. longicolle were incorrectly identified and should be called T. retusum. He said that Dujardin found the worms which he described in the cecum of the chicken and called them T. longicolle but Railliet thought the diagnosis given by Dujardin was in flagrant contradiction to that of Rudolphi. The writer has found no statement in Dujardin's paper concerning the portion of the digestive tract from which he obtained his specimens. He merely named the various hosts in which he found them, although he did mention that Schrank found the worms in the "rectum" of the chicken and Froelich found them in the intestine of the pheasant and that he obtained specimens from both of these hosts.

Since Dujardin did not state definitely whether he obtained his specimens from the intestine or the cecum of the birds there seems to be little justification for Railliet's conclusion that he was dealing with Trichosome retusum Railliet, 1893. On the contrary, the difference in egg size as given for the worms obtained by the two investigators (Railliet's measured 50-55μ long by 30-32μ wide; Dujardin's were 65μ long by 23μ wide) and the fact that Dujardin mentioned only

one bacillary band whereas Railliet found three, indicate that they were probably dealing with different species. As a matter of fact, Dujardin mentioned the funnel-shaped appendage with which the vulva of the female was provided thus suggesting that he may have been working with the same species of worm described by Molin (1858) as Calodium caudinflatum. Railliet (1895), in a description of T. retusum, reported no such appendage. On the contrary, he wrote: "vulva situated a little distance behind the origin of the intestine, slightly prominent, transverse, without true appendage".[1] However, Morgan (1932) reported that a membranous appendage is occasionally present at the vulva in immature specimens of Capillaria retusa. Such a structure may not be a true appendage but merely a prolapsed oviduct.

Several workers prior to 1819 had given sketchy and incomplete descriptions of nematodes which were probably "trichosomes" and for which they proposed species names. As previously mentioned, Dujardin (1845) gave a list of the names which he considered as possible synonyms of the same species described by him under the name Trichosoma longicolle, Rudolphi, 1819, some of which had been previously listed and discussed by Rudolphi (1819). However, Hassal (1896) in a check-list of animal parasites of chickens gave the most complete list under the name Trichosoma longicolle with synonyms as follows:

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[1]Railliet, A. Traité de zoologie médicale et agricole. Paris. 1895. p. 484. (Translation by N.F.M.).

Trichosoma longicolle Rudolphi, 1819[1]

? 1782 Gordius gallinae Goeze
? 1790 Filaria gallinae (Goeze, 1782) Gmelin
? 1791 Filaria phasiani Froelich
? 1796 Linguatula unilinguis Schrank
? 1802 Filaria tetricis Froelich
? 1803 Capillaria semiteres Zeder
? 1810 Hamularia nodulosa Rudolphi
longicolle Rud. of Dujardin and Eberth (nec Rudolphi) see T. retusum.

The writer has not obtained sufficient information concerning the above names to voice an opinion regarding the majority of them although T. longicolle Rud. of Dujardin and Eberth has already been discussed. Let it be mentioned, however, that for one reason or another these questionable synonyms have been unacceptable to the various taxonomists who have dealt with this group.

Because of the brevity of the description given by Rudolphi and the consequent lack of definite characters necessary to identify the worms which he found, it is suggested that Trichosoma longicolle Rudolphi, 1819 should be included in the list of synonyms given above. The entire list should then be referred to Capillaria caudinflata (Molin, 1858).

In view of the information presented in the foregoing discussion, the following classification is suggested for the nematode reported in this paper:

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[1]Hassal, A. Checklist of animal parasites of chickens (Gallus domesticus). Circular (9), Bur. An. Ind., U. S. Dept. Agric. 1896. p. 6.

Phylum--Nemathelminthes Vogt (quoted by Carus, 1863).

Class--Nematoda Rudolphi, 1808, emend. Diesing, 1861.

Order--Trichurata Skrjabin, 1916.

Super-family--Trichinelloidea Hall, 1916.

Family--Trichuridae Travassos, 1915.

Sub-family--Capillariinae Railliet, 1915.

Genus--Capillaria Zeder, 1800.

species--caudinflata (Molin, 1858).

The literature on Capillaria caudinflata (=T. longicolle) has been written, for the most part, by European investigators. However, 4 papers by North American helminthologists, have thus far been found where Capillaria caudinflata or its synonym Capillaria longicollis is mentioned. Stiles and Hassal (1894), in a catalog of the animal parasites in the collection of the Bureau of Animal Industry and their own private collections stated that Trichosoma longicolle was collected by Stiles from Tetrao urogalli at Leipsic. Hassal (1896) again listed Trichosoma longicolle Rud. in a check list of animal parasites of chickens but neither of these papers referred to the presence of this species in the United States. Beach and Freeborn (1936), in a discussion of poultry nematodes in California, considered Capillaria longicollis and C. caudinflata as separate species although they did not report any specific identification of capillarid worms found in the lower digestive tract of chickens in that state. Morehouse (1939) reported that Capillaria caudinflata was found in the intestine of chickens received from Iowa,

Minnesota, Ohio, Illinois, Wisconsin, Pennsylvania, Missouri, Kansas, Indiana and Michigan.

Among the papers by European authors, those of Rudolphi (1819), Dujardin (1845), Molin (1858), and Eberth (1863) have already been discussed. Diesing (1851), in his Systema Helminthum, included Trichosoma longicolle in his list of species inquirendae. Diesing (1861a) and again (1861b) entered Calodium caudinflatum Molin, 1858, but made no further reference to T. longicolle. According to Shipley (1909), Kowalewski (1894) described Trichosomum gallinum from the fowl but later (1901) described and figured the same species under the name Trichosoma caudinflatum. The writer has been unable to examine Kowalewski's papers.

Shipley (1909), an English author, found capillarids in the red grouse Lagopus scoticus which he obtained from Perthshire. He identified the worms as Trichosoma longicolle Rud. and listed Calodium caudinflatum Molin, Trichosoma gallinum Kowal., and Trichosoma caudinflatum Kowal. as synonyms. Shipley's paper was the earliest comprehensive treatise of this species of nematode. His description and figures make it almost certain that he was dealing with the same nematode found by the writer in chickens of the United States although it must be remembered that he obtained his specimens from an entirely different species of gallinaceous birds. On the other hand, host-specificity, as a general rule, seems to be of little

taxonomic value when dealing with the capillarids. Experiments reported in this paper show it to be lacking in Capillaria caudinflata.

Morgan (1932), another English author, found some nematodes in the English domestic fowl which he identified as Capillaria longicollis (Rud., 1819) Travassos, 1915. He listed as synonyms: Trichosoma longicolle Rud., 1819; Calodium caudinflatum Molin, 1858; Trichosoma gallinum Kowal., 1894; and Trichosoma caudinflatum Kowal., 1901. Although Morgan considered his specimens under the name Capillaria longicollis, he was by no means certain that he was using the correct nomenclature.

Clapham (1935), a third English author, reported Capillaria longicollis from 44 out of 380 birds examined. She stated that it is a common parasite of the small intestine of many gallinaceous birds but concluded that the worms usually seem to occasion no damage. It should be noted that the largest number of worms which she found in a single bird was 23 and usually only 3 or 4 which is a very light infection according to the writer's experience with chickens of the United States. Clapham also reported that she had previously obtained C. longicollis from the pheasant, Phasianus colchicus.

Others who have found this nematode parasite are Huus (1931) who reported Capillaria longicollis from the Norwegian ptarmigan, Lagopus lagopus, and Mönnig (1933) who found this

species in the small intestine of chickens of Utrecht. On the contrary Reis et. al. (1936) stated that C. longicollis is not found at São Paulo, Brazil.

The lower portion of the digestive tract of the chicken (i.e. the portion posterior to the gizzard) is parasitized by several species of Capillaria other than C. caudinflata. Rudolphi (1819) described the pigeon capillarid which is now known as Capillaria columbae. This nematode is frequently found in the small intestine of chickens and enjoys a wide geographical distribution as a parasite of the latter host.

Von Linstow (1873) described another capillarid from the intestine of the chicken which he called Trichosoma collare. However, Morgan (1932) suggested that the worms described by Von Linstow may have been the same as those described by Railliet (1893) under the name Trichosoma retusum. If such is the case, then the name of Von Linstow's species becomes a synonym of Capillaria retusa (Railliet, 1893). Both species have been described as having annular constrictions in the cuticula near the anterior end and both are said to have spiny spicule sheathes. On the other hand, Capillaria collaris (v. Linstow, 1873) was described as having 2 lateral bacillary bands whereas Capillaria retusa has a third broad ventral band. The fact that Von Linstow found his specimens in the intestine of the chicken while Railliet found his in the cecum also constitute another point of difference.

Capillaria gallinum (Kowal., 1894), as previously mentioned, was originally described from the intestine of the chicken but has since been made a synonym of C. caudinflata. Kowalewski (1894) described another species from the cecum of the chicken which he called Trichosoma dubium. This name is now considered a synonym of Capillaria retusa.

/More recently (1934) a species of capillaria was described by Teixeira de Freitas and Lins de Almeida from the small intestine of chickens at Rio de Janeiro, Brazil. The chief distinguishing characteristic of this new species which they called Capillaria bursata is the morphology of the caudal extremity of the male. It was described as having caudal alae and a membranous copulatory bursa supported by 4 papillae, 2 of which are curved ventrally terminating in the margin of the bursa, the other 2 being latero-dorsal in position.

From the foregoing discussion, we may conclude that 4 and possibly 5 distinct species of capillaria are known to occur in the lower digestive tract of Gallus domesticus L.; namely, Capillaria caudinflata (Molin, 1858), C. columbae (Rudolphi, 1819), C. bursata Teixeira de Freitas and Lins de Almeida, 1936, and possibly C. collaris (v. Linstow, 1873), all of which occur in the small intestine; the only species thus far found in the cecum is C. retusa (Railliet, 1893).

Reports of the successful experimental transmission of worms belonging to the genus Capillaria to avian hosts are few, indeed. Cram (1931) was able to transmit Capillaria contorta,

a capillarid occurring in the upper digestive tract of numerous birds, to a quail and a domesticated duck by feeding embryonated eggs obtained from a European pheasant. She was, however, unable to transmit this species to chickens, but later (1936) reported its successful experimental transmission to turkeys.

In 1931, Cram also reported the transmission of another capillarid, C. retusa, which occurs in the lower digestive tract of various birds. She stated that she was able to produce an infestation in a chicken and a quail by feeding a 42-day old culture of embryonated eggs collected from a hungarian partridge. On the other hand, Levine (1938) stated that Cram (1937--personal communication to Dr. Levine), after a re-study of her specimens, is convinced that she was working with C. columbae and not C. retusa. Therefore, it appears that the experimental transmission of C. retusa still remained undemonstrated.

Capillaria columbae is perhaps the easiest species to transmit since the period of embryonation is short and no intermediate host is required. Levine (1938) confirmed the direct life-cycle of this species which was later worked out in detail by Wehr (1939).

All efforts of various investigators to transmit embryonated eggs of Capillaria annulata by direct feeding have been unsuccessful. However, Wehr (1936) showed that earthworms of the species Helodrilus foetidus and Helodrilus caliginosus serve as intermediate hosts to this parasite.

Due to the evident variation in method of transmission among the capillarids, it would seem unwise to postulate any particular life-cycle for a given species. It is apparent that the life-cycle must be worked out individually for each species.

EXPERIMENTAL

A. Material and Methods

The capillaria worm eggs used in these experiments were obtained from infected chickens originating in various parts of the United States. To prepare a culture of eggs, the droppings of one or more infected birds were collected and washed through a fine-mesh milk strainer screen in order to remove the coarse particles. The washings were then allowed to settle and the liquid was decanted leaving the sediment containing the droppings at the bottom of the beaker. In some cases this process was repeated 2 or 3 times, depending upon the amount of sediment present. A centrifuge equipped with 50 cc. centrifuge tubes was used to remove excess water from the sediment. The eggs were then suspended in a saturated solution of sodium chloride by centrifugation and the salt water was poured off, diluted to about 5 times its volume with tap water and the eggs were then concentrated in the bottom of a centrifuge tube. By repeating the salt-flotation process a culture of eggs could be obtained which was practically free of fecal material. Although this procedure did not remove coccidia oocysts, Ascaridia and other roundworm eggs or onchospheres of Hymenolepis sp., their presence was of no

great consequence to the investigation. The methods used for embryonation of capillaria worm eggs have been fully discussed elsewhere in this paper.

For the high temperature experiments an electrically operated, thermostat controlled incubator was used and for the low temperature experiments a Crosley household refrigerator was employed.

All chicks used in the transmission experiments were hatched in the laboratory and were kept in wire bottom cages from the time of hatching until the termination of the experiment, except as otherwise noted. Various breeds of chickens were used in the experiments, the kind used in any given experiment usually being determined by the availability of the birds at that particular time although a preference for the heavier breeds prevailed since the latter are easier to handle in cages than the Mediterranean breeds.

Unless otherwise noted in the text, the grasshoppers, confused flour beetles and Tenebrio molitor used in the intermediate host tests were laboratory raised. All other arthropods as well as the earthworms were collected from chicken runs and other nearby locations.

The measurements of worms and worm eggs, as recorded in this paper, were made with an ocular micrometer or a camera lucida. All photographs were time exposures taken with the aid of a Bausch and Lomb microscope and an Eastman Senior 620 kodak or a view camera using cut film.

The material for the geographical distribution studies was obtained in different ways. The principal source of specimens was through the diagnosis laboratory of Dr. Salsbury's Laboratories which receives chickens from all parts of the United States. A second source of specimens was the purchase of chickens for experimental purposes from various poultry raisers and produce dealers throughout the country. All specimens from whatever source were received and identified by the writer.

Microscope slides were made of many specimens using various techniques but the best results were obtained by placing the specimens in glycerine jelly and mounting them between two cover-glasses, one of which was about 2 mm. smaller than the other. The cover-glasses were then mounted on a microscope slide with the smaller cover-glass toward the balsam.

B. The Geographical Distribution of Capillarids of the Lower Digestive Tract

A preliminary report on the geographical distribution of 3 species of capillarids, C. caudinflata, C. columbae and C. retusa, found in the lower digestive tract of chickens was made by Morehouse (1939). Having continued this study, a complete report is made at this time. One or more capillarid species have been found in chickens received from 56 counties located in 17 different states (Table 1). A total of 1678

Table 1. Geographical Distribution of Capillarids of the Lower Digestive Tract

State	County	No. of Birds	C. caudinflata		C. columbae		C. retusa	
			Males	Females	Males	Females	Males	Females
Alabama	Jefferson	1					9	20
Georgia	Ware	1			11	10		
Illinois	Adams	12	121	237	5	11		
	Bond	1	11	23				
	Lake	1		3	3	15		
	Stark	1	7	33				
	Stevenson	1	7	3				
Indiana	Jackson	1			41	32		
	Sullivan	2	1	8				
Iowa	Bremer	2	1	4				
	Buena Vista	1		1				
	Butler	1		3				
	Cerro Gordo	1		4				
	Chickasaw	2	31	134			2	4
	Floyd	6	12	101			1	1
	Hancock	2	4	52				
	Howard	2	2	1				
	Mitchel	2	1	27				
	Muscatine	1		4				
	Palo Alto	1		1				
Kansas	Brown	1	6	11				
	Miami	1		1				
Kentucky	Henderson	1		1				
Maryland	Wicomico	1			16	11		
	Baltimore	1			2	45		
Michigan	Bay	2	84	158				
	Ottowa	1	4	7				

Table 1. (continued)

State	County	No. of Birds	C. caudinflata		C. columbae		C. retusa	
			Males	Females	Males	Females	Males	Females
Minnesota	Fairbault	3		14				
	Freeborn	1		8				
	Le Sueur	2			38	159		
	McLeod	1		2				
	Norman	2			116	230		
	Stearns	2	2	14				
	Waseca	2			1	10		
Missouri	Jefferson	1		2				
	Linn	1		6				
	Nodaway	4	9	12				
	St. Louis	?	1	12				
	Perry	1		2				
New York	Cortland	1		23				
	Otsego	1			16	103		
	Wayne	1		4				
Ohio	Cuyahoga	1	16	85				
	Hardin	1		1				
	Putnam	1		3				
	Richland	1		2		3		
	Shelby	1			1	11		
	Stark	1					1	7
Pennsylvania	Union	1	1			4		
	Somerset	1			95	209		
Rhode Island	Providence	1				11		
West Virginia	Marion	1	4	11		2		
Wisconsin	Brown	1				1		
	St. Croix	1			4	13		
	Dunn	1	85	249				
	Trempealeau	1		1	31	49		

C. caudinflata, 1309 C. columbae and 45 C. retusa were collected and identified from approximately 90 naturally infected chickens received at Dr. Salsbury's Laboratories, Charles City, Iowa. Of these, 410 (24.43%) C. caudinflata, 380 (29.02%) C. columbae and 13 (28.88%) C. retusa were males. The largest number of capillarids found in a single bird was as follows: C. caudinflata 334, C. columbae 346 and C. retusa 29. Six of the birds were host to both C. caudinflata and C. columbae.

C. Life-Cycle

1. The exogenous development of Capillaria caudinflata

The eggs of Capillaria caudinflata are spindle-shaped, slightly yellowish in color having thick punctate shells provided with a transparent opercular plug at each end. Although there is some variation in the size and shape of the eggs of this species, they are a little narrower in proportion to their length than are the eggs of Capillaria columbae. Dujardin (1845) stated that the eggs of C. longicollis (=C. caudinflata) measure from 61μ to 65μ long by 23μ wide and Morgan (1932) found the average measurements to be 53μ long by 23μ wide.

The writer has found that 25 eggs measured by means of an ocular micrometer varied from 50μ to 59μ in length and from 21μ to 24μ in width. The mean size was 55μ by 23μ. All measurements were made without the use of a cover-glass in order to avoid distortion of the eggs due to pressure.

The eggs of Capillaria caudinflata are passed in the droppings of infested chickens in an unsegmented condition (Plate I, Fig. 1). Early attempts to embryonate these eggs in 2% formaldehyde solution met with failure. Consequently another bacterioide, 2.5% potassium dichromate, was substituted with much better results. It was found that by using this medium approximately 100% embryonation could be obtained in the cultures. This appears to be in sharp contrast to the results reoprted by Levine (1936), who attempted to embryonate eggs of the pigeon capillarid in 2% potassium dichromate but found that many of the embryos died before embryonation was completed. Cram (1936) found that weak solutions of formalin and potassium dichromate were unsuitable as embryonating media for capillarid eggs. Although Wehr (1939) reported the use of tap water or distilled water for the embryonation of the eggs used in his experiments with Capillaria columbae, he stated that eggs embryonated in 1 to 2 percent formalin contained fully formed embryos as early as those cultured in tap or distilled water. The writer has also found this to be true in the case of C. columbae whereas eggs of C. caudinflata failed to develop properly, thus indicating a greater susceptibility of the latter species to the action of formaldehyde. Cultures of C. caudinflata eggs for the early part of this investigation were embryonated in 2.5% potassium dichromate, although 1% nitric acid and, more recently, tap water has been used with good success.

PLATE I.

Embryonation of *Capillaria caudinflata* Eggs

Fig. 1. Freshly passed *C. caudinflata* egg. Note the translucent equatorial spot.

Fig. 2. *C. caudinflata* egg showing the first cleavage line which is formed during the first 24 hours of development.

Fig. 3. *C. caudinflata* egg showing 2 cleavage lines. The second cleavage is usually completed in the 36-hour old egg.

Fig. 4. *C. caudinflata* egg showing 3 cleavage lines. The division of the central cell to produce the 4-cell stage is atypical.

Fig. 5,6. *C. caudinflata* egg after 45 hours of development. The large blastomeres are approximately 8 in number.

Fig. 7. *C. caudinflata* egg after 70 hours of development. The small blastomeres have a peripheral arrangement.

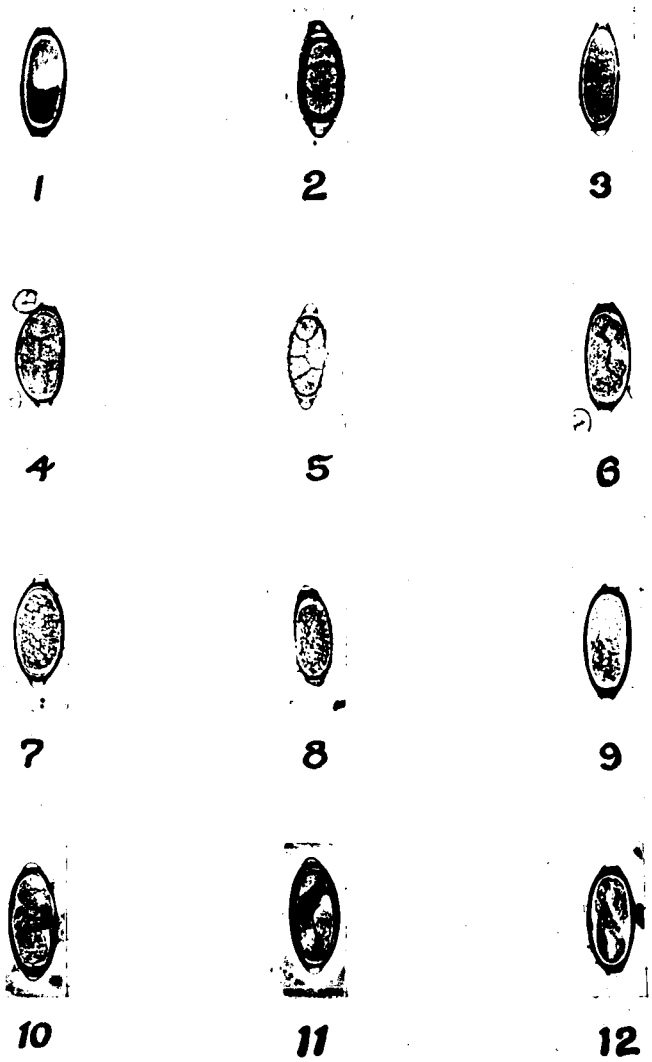
Fig. 8. *C. caudinflata* egg after approximately 80 hours of development. Compare with Fig. 7 noting that the embryo has shrunken away from the egg shell. The embryo is non-motile at this stage.

Fig. 9. *C. caudinflata* egg observed in a 100-hour old culture. Note the translucent end of this bean-shaped embryo.

Fig. 10. *C. caudinflata* embryo in the S-shaped stage. Elongation of the embryo occurs rapidly.

Fig. 11,12. Mature *C. caudinflata* embryos photographed after 11 days of development.

PLATE I.



1 2 3

4 5 6

7 8 9

10 11 12

A single comparative test on the value of 2.5% potassium dichromate solution and tap water for use in embryonating C. caudinflata eggs was run on a divided culture from one infested bird. Since 94% of the eggs in potassium dichromate solution and 92% of the eggs in tap water became embryonated, there is apparently little difference in the value of these two media.

a. The embryonation of the ova. The contents of freshly passed unsegmented eggs of C. caudinflata have a uniformly granular appearance except for a round or flask-shaped equatorial spot which (Plate I, Fig. 1) is possibly associated with the fertility of the ovum although it has often been observed in eggs which fail to undergo segmentation.

The incubation period of the eggs of C. caudinflata is somewhat longer than that of C. columbae in which species the eggs may, under favorable conditions, become embryonated in 6 days. The following observations on the development of C. caudinflata eggs were made on a culture collected from droppings passed during a 5-hour period. After these eggs were freed from the droppings by salt flotation and centrifugation, they were placed in petri dishes in a shallow layer of tap water and were held at a room temperature of 78° ± 4°F. The majority of the eggs had undergone their first cleavage at the end of 24 hours (the incubation time as stated in this report is figured from the beginning of

the 5-hour collection period) but none had yet undergone the second cleavage. Not until 5 hours later were any eggs observed in the 3-cell stage.

The first and second cleavages which occur very regularly in these eggs are total and unequal. The first cleavage line runs transversely resulting in a new cell containing a little less than one-third of the contents of the egg (Plate I, Fig. 2). The second cleavage line appears in a similar position toward the opposite end of the egg, resulting in 2 polar cells of approximately the same size with a somewhat larger cell lying between them (Plate I, Fig. 3). No prediction can be made as to where the third cleavage line will occur. As a rule, the first cell to be formed divides to produce the fourth cell but in some cases it may be formed by division of the center cell (Plate I, Fig. 4). From this stage of development, cleavage proceeds without any apparent sequence or rhythm.

Examination of eggs 45 hours after the beginning of the collection period showed the majority of them to be in the 8-cell stage although one egg was found to contain 14 cells. At the 8-cell stage the blastomeres are rather large (Plate I, Figs. 5,6). When the culture was examined after 54 hours of incubation, it was difficult to accurately determine the number of cells. However, the majority of the eggs appeared to contain morulae made up of small blastomeres. A very

close estimate of the number in one egg showed that it was in approximately the 20-cell stage.

Up to this time, no characteristic arrangement of the cells within the egg was observed but as development proceeded beyond the 20-cell stage, a peripheral arrangement of the blastomeres occurred and there was a more or less sharp delimitation of the peripheral cells from a central cluster (Plate I, Fig. 7). This arrangement appeared quite definite in eggs which had been incubated for 70 hours. Thus far, the blastomeres had retained a uniformly granular appearance and had occupied virtually the entire area within the egg shell, but eggs examined after 120 hours of incubation showed a tendency for one end of the embryo to shrink away from the inner surface of the egg shell (Plate I, Fig. 8). The shrinking which proceeded so slow that no motion could be observed, produced a space of considerable size between the embryo and the inner surface of the shell. Eggs observed after 173 hours of incubation did not contain actively motile embryos but protoplasm at the shrunken end had become less granular and the margin in this region had assumed a truncate appearance. The transformation into vermiform embryos then proceeded rapidly. An elongation of the embryo, occurring chiefly at its granular end, again caused the embryo to fill the space within the egg shell. As the embryo continued to elongate, the first indication of active motility was observed.

The embryo, somewhat vermiculate in appearance became successively bean-shaped, (Plate I, Fig. 9), S-shaped, (Plate I, Fig. 10) and U-shaped. The motility of the embryo was, for a time, confined largely to the granular end of the larva but later the entire worm was seen thrashing about within the egg shell. The larvae remain very active for several days or even weeks, but it has been observed that the worms in old cultures are rather quiescent. The method whereby the transformation into a vermiform embryo takes place is not quite clear. Elongation of the embryo plays a definite part but it is also possible that there is a splitting of part of the cells at the granular end, away from the remainder of the embryo. At any rate, a few of the eggs examined after 197 hours of incubation contained U-shaped embryos while some were still in the bean-shaped stage, others were in the S-shaped stage and a few had not yet reached any of these developmental stages. Moulting of the larvae within the egg-shell has been used in some species of nematodes to indicate maturity but no such moulting has been observed in C. caudinflata. However, coiled embryos resembling those which had been kept for several months were observed after 262 hours of incubation (Plate I, Figs. 11, 12).

The above observations show that the eggs of C. caudinflata, suspended in tap water and held at a room temperature of $78^{\circ} \pm 4^{\circ}$F., appear to become fully embryonated in approximately

11 days. The first indication of active motility was observed on the eighth day of incubation. Since it is not known whether the above conditions were optimum for development it is possible that other conditions might shorten the period of development to some extent. However, experiments which are reported elsewhere in this paper show that extremely low temperatures and higher temperatures have an adverse effect upon development.

(1) The effect of environmental factors on eggs of C. caudinflata

A series of tests was conducted in order to determine the effect of high and low temperatures, and of certain chemicals upon unembryonated and embryonated eggs of C. caudinflata.

All eggs were recovered from the droppings in the manner previously described. If tests were to be run on unembryonated eggs, the eggs were immediately subjected to the environmental condition under consideration. If, on the other hand, the tests were to be run on embryonated eggs, the freshly collected eggs were placed in 2.5% potassium dichromate or tap water and were held at room temperature until motile embryos were present.

(a) The effect of high temperature on unembryonated eggs.

Experiment 1. A culture of eggs of C. caudinflata suspended in 2.5% potassium dichromate solution was placed in an incubator where the temperature variation was $40^{\circ} \pm 2^{\circ}$C. Examination of this culture from time to time showed that the eggs were developing,

but at a much slower rate than a control culture of eggs held at room temperature. Unfortunately this culture was allowed to dry up 107 days after it was placed in the incubator. Water was added and the eggs were examined but none was found which had developed to the larval stage.

Experiment 2. Another experiment was carried out where half of a culture of C. caudinflata eggs was suspended in 2.5% potassium dichromate solution and placed in an incubator held at 40° ± 2°C. The other half, also a potassium dichromate culture, was held at room temperature. Examination of 25 eggs from each of these cultures 31 days later showed that none of the eggs in the incubator had developed beyond the morula stage, some had never developed at all, while others were in the 2 and 3 cell stage. In the control culture, held at room temperature, 100% of the eggs examined contained coiled embryos, many of which were motile.

Experiment 3. A culture of C. caudinflata eggs was collected from droppings passed by an infected bird during a 30 hour period. The culture was placed in tap water and was divided into 2 equal portions. Half of the culture was held at room temperature and half was placed in an incubator where it remained for 42 days. The temperature variation in the incubator during the first week as recorded by a thermograph was 39°C. to 42°C. During the second week the contact points on the thermostat stuck together 3 times and ran the temperature up as high as 46°C. At no time during the entire 6 weeks was

the temperature below 38°C. Examination of 100 eggs from the incubated culture at the time of removal to room temperature showed that only a few eggs had undergone cleavage and that the majority of them showed signs of degeneration. None had reached the coiled embryo stage. Of 25 eggs examined from the control culture held at room temperature, 100% contained coiled embryos.

Experiment 4. A culture of C. caudinflata eggs was collected from droppings passed by an infected bird during a 24-hour period. The culture was placed in tap water and was divided into 2 equal portions. One portion was held at room temperature and the other was placed in an incubator where it remained for 23 days. The temperature variation in the incubator for this period, as recorded by a thermograph, was 38°C. to 42°C. When the culture was removed from the incubator, 100 eggs were examined. A few of them had divided 2 or 3 times but most of them had failed to develop at all. None had reached the coiled embryo stage. In the control culture held at room temperature, 12% of the 25 eggs examined contained coiled embryos while many others were in the late morula stage of development.

(b) The effect of low temperature on unembryonated eggs.

Experiment 1. A culture of eggs of C. caudinflata was obtained from the droppings of an infected chicken and was divided into 2 portions. One portion was placed in a 2.5%

solution of potassium dichromate and was held at room temperature; the other portion was suspended in distilled water and was placed in the freezing unit of a household refrigerator where the temperature was maintained at about -10°C. This culture remained in the refrigerator for 14 days, when it was removed and placed in a 2.5% potassium dichromate solution and was held at room temperature. Examination of the culture at the time of removal showed that none of the eggs had begun to develop whereas eggs of the control culture contained morulae with small blastomeres. Examination of the refrigerated culture one week after removal to room temperature showed that a few of the eggs were developing. After 44 days incubation it was found that 96% of the eggs of the unrefrigerated control culture contained mature coiled embryos. In the refrigerated culture examined at the same time, no eggs were found which had developed beyond the late morula stage. When the refrigerated culture was again examined 84 days from the beginning of the experiment, 21% of 100 eggs observed contained coiled mature embryos; all other eggs appeared to be degenerate.

This test shows that a temperature of -10°C. for a period of 2 weeks is lethal to many of the unembryonated eggs of C. caudinflata but approximately one-fifth of them survived and were capable of development.

Experiment 2. A culture of eggs of C. caudinflata in tap water was placed, immediately after collection, into the freezing unit of a refrigerator where the temperature was about -10°C. This culture remained frozen for 33 days with one exception when the refrigerator was defrosted over night. On the 33rd day the culture was removed to room temperature and potassium dichromate solution was added. The eggs were examined at the time of removal but none of them had undergone any cleavage. When the culture was 127 days old, 58% of the eggs contained coiled embryos while 42% had not developed at all.

Experiment 3. A culture of C. caudinflata eggs was collected from droppings passed by an infected bird during a 24 hour period. This culture was placed in tap water and was divided into 2 parts. One part was kept at room temperature and the other was placed in the bottom of the refrigerator where it was held at approximately 6°C. for 39 days. At the time this culture was removed to room temperature, the examination of 50 eggs showed that only 8% of them had developed at all. None of them had developed beyond the 3-cell stage. On the other hand, the control culture held at room temperature showed 92% embryonation 27 days after it was collected.

Experiment 4. A culture of C. caudinflata eggs was collected from droppings passed by an infected bird during a 24 hour period. This culture was placed in tap water and was

divided into 2 equal parts. One part was kept in the bottom of a refrigerator where the temperature was maintained at approximately 6°C. for 37 days. The other portion of the culture was held at room temperature to serve as a control. When the culture was removed from the refrigerator only 12% of the 50 eggs examined had divided and none had developed beyond the 3-cell stage. Of 50 eggs examined at the same time from the control culture, 82% contained coiled embryos.

(c) The effect of low temperature on embryonated eggs of C. caudinflata

Experiment 1. A culture of C. caudinflata eggs was collected and allowed to embryonate in 2.5% solution of potassium dichromate at room temperature. Thirty-five days after collection the culture was freed of potassium dichromate by repeated centrifugation and was placed in a refrigerator where it remained for 21 days at a temperature of about -10°C. The culture was then removed to room temperature and potassium dichromate solution was added. At the time of removal from the refrigerator there were no signs of degeneration in the eggs but 18 days later many of them had a vacuolated appearance. Examination of the culture when it was 171 days old showed that 70% of the eggs showed degenerate embryos, 9% had never developed or had disintegrated completely, and 21% contained embryos which appeared to be normal although no motility was observed. In the absence of motility and infectivity tests it cannot be definitely stated whether or not these

embryos were alive. In a culture which had been maintained for 164 days at room temperature, 96% of 50 eggs examined showed no indication of degeneration.

Experiment 2. Having discovered that earthworm digestive juice causes mature C. caudinflata larvae to hatch, this phenomenon was utilized in the following experiment for testing the viability of embryonated eggs subjected to low temperature.

Eggs from a 17-day old C. caudinflata culture were treated with freshly collected earthworm digestive juice. Hatching of many larvae followed after several minutes. On the following day the culture was divided into 3 parts. Part 136A was placed in the ice tray of an electric refrigerator where the temperature was -10°C; part 136B was placed in the bottom of the refrigerator at a temperature of 6°C. and part 136C was held at room temperature. Six days later each culture was tested for viability by treatment with earthworm digestive juice. Hatching occurred in all 3 samples. On the 14th day of refrigeration, eggs held in the freezing chamber failed to hatch or to become motile within the egg shell, although many larvae hatched from parts 136B and 136C when treated with the same sample of earthworm digestive juice. The same results were obtained when the 3 cultures were treated with fresh samples of earthworm digestive juice on the 2 following days.

(d) The effect of out-door winter temperature upon embryonated and unembryonated Capillaria caudinflata eggs

Tests had previously been run on the effect of low temperature on C. caudinflata eggs where the temperature was held relatively constant. The following experiments were conducted in order to get observations on the effect of low temperature where the conditions more nearly approximated those to be found in nature.

Experiment 1. In order to test the effect of winter temperature on unembryonated eggs, a culture of C. caudinflata eggs was collected on January 26 and placed in tap water. This culture was divided into 2 equal parts, one part was held in the laboratory at room temperature and the other was placed on a window ledge outside the building where it remained for 64 days. The extremes of temperature during this period, as recorded by the local federal weather observer, were a low of -21°F. recorded on February 25th and a high of 68°F. recorded on March 30. The mean temperatures for February and March were 21.7°F. and 28.3°F. respectively. All of the 50 eggs examined from the latter culture when it was brought in from the outside were considered degenerate since the protoplasm appeared vacuolate. No indication of development was observed in any of the eggs. However, in the control culture held at room temperature, coiled embryos were found in 96% of the 50 eggs observed.

Experiment 2. A culture of C. caudinflata eggs collected on February 21 from droppings passed during a 24-hour period by an infected bird was treated in the same way as the culture of the preceding experiment. The culture was divided and half was placed in the room while the other half was placed on the window ledge where it remained for 44 days. The extremes of temperature for this period, as recorded by the local federal weather observer, were a low of -21°F. recorded on February 25th and a high of 68°F. recorded on March 30th. When the exposed culture was removed to room temperature, none of the 50 eggs examined had undergone segmentation, all appearing degenerate as indicated by the vacuolate protoplasm. On the other hand, 94% of the 50 eggs examined from the control culture held at room temperature contained coiled embryos.

Experiment 3. In order to test the effect of winter temperature on embryonated eggs of C. caudinflata a 100-day old culture of eggs was freed of the potassium dichromate embryonating medium and was brought into tap water. Since the eggs of this culture contained mature normal appearing embryos, the culture was divided into 2 equal parts on February 2nd. One part was placed in the laboratory where it was held at room temperature and the other part was placed on a window ledge just outside the building where it remained for 63 days. The extremes of temperature during this period, as recorded by the local federal weather observer, were a low of -21°F. recorded on February 25th and a high of 68°F. recorded on

March 30th. The mean temperatures for February and March were 21.7°F. and 28.3°F. respectively. Of 50 eggs examined from the test culture at the time of removal to room temperature, 98% contained coiled embryos which showed no signs of degeneration. No means were available at the time these data were obtained for ascertaining the viability of the larvae but no motility could be detected. In the control culture all of the 50 eggs examined contained coiled embryos which appeared to be normal.

(e) The effect of formaldehyde on unembryonated eggs

As previously mentioned, the writer found that 2% formalin was not favorable to the embryonation of eggs of C. caudinflata. Therefore experiments were run to determine the extent of the damage to the eggs.

Experiment 1. A culture of eggs was divided into 2 equal parts, half of which was placed in 1% formalin and half in 2.5% potassium dichromate to serve as a control. Both cultures were held at room temperature. Twenty-three days later 25 eggs were examined from each culture. In the formalin culture none had passed the late morula stage of development whereas 18 of the eggs in the potassium dichromate culture contained active coiled embryos. Nine days later 100 eggs were examined from each culture. In the formalin culture 16% of the eggs contained coiled larvae while 100% of the eggs in the control culture had mature embryos. The formalin

culture was kept for 5 months and was examined from time to time but the greatest percentage of mature embryos observed at anytime was 18% found on the 49th day of the experiment.

Experiment 2. A culture of C. caudinflata eggs was obtained from droppings passed by infected birds within a 24 hour period. The culture was divided into 3 equal parts, one portion being placed in tap water, another in a 1% formalin solution and the third in a 2% formalin solution. All 3 cultures were held at room temperature. One hundred eggs were examined from each of these cultures 42 days later. In the tap water culture 92% of the eggs contained coiled embryos. None of the eggs in the 1% formalin culture had developed farther than the 5-cell stage while more than 50% of them had never divided. In the 2% formalin culture no eggs were found past the 2-cell stage, 96% of them showing no cleavage lines at all.

2. Experiments on the transmission of Capillaria caudinflata to chickens

It has already been pointed out that life-cycles of 2 distinct types are known among the members of the genus Capillaria. The first transmission experiments carried out in this investigation were the direct administration of embryonated C. caudinflata eggs to chickens.

a. Experiments on the direct transmission of C. caudinflata to chickens

Experiment 1. Eggs of C. caudinflata collected Dec. 2, 1938 from a chicken obtained from Kansas City, Mo. were allowed to

embryonate in 2.5% potassium dichromate solution. When the culture was 43 days old, a portion of the embryonated culture was given to a barred rock chick. The droppings were then examined daily by salt flotation, with few exceptions, for a period of 53 days but no eggs were found. Post-mortem examination of the chick 3 days later showed that it had not become infected with capillaria worms.

Experiment 2. In order to detect a capillaria infection, if present, the droppings of 6 barred rock chicks approximately 3 weeks old were examined by the salt flotation technique every day, with one exception, for a period of 2 weeks. No capillarid eggs were found. On January 31, 1939, chick No. 102 was given a culture of C. caudinflata eggs collected November 4, 1938. These eggs contained numerous mature embryos. On the same day chicks No. 103, No. 104, No. 106 and No. 107 were given a different culture of embryonated C. caudinflata eggs. The latter culture collected on 2 different dates, one part 62 days old and the other 57 days old, had been embryonated in 2.5% potassium dichromate solution. Chick No. 105 was given a culture which had been frozen for several days and later embryonated in 1% nitric acid at room temperature. The eggs contained well formed embryos but no motility was observed at the time of infection. The droppings were examined by salt flotation every 2nd or 3rd day for varying periods of time as follows: No. 102--36 days,

No. 103--36 days, No. 104--24 days, No. 105--36 days, No. 106--32 days and No. 107--25 days. Post-mortem examination of the digestive tracts of these chicks at the end of their respective examination periods showed that none of them had become infected with capillaria worms although one chick, No. 104, was host to one male cecal worm, Heterakis gallinae, the egg having been given with the capillaria egg culture.

Experiment 3. Three battery raised chicks were given embryonated eggs of C. caudinflata. Chick No. 1 received a 6-weeks old culture of eggs which had been embryonated in 2.5% potassium dichromate solution and later removed to tap water. Since potassium dichromate is somewhat toxic to chicks, the cultures used in all previous experiments were washed free of the dichromate solution and brought into tap water just before administration to the chicks. In order to eliminate the possibility of such a change effecting the eggs adversely, a culture embryonated in 2.5% potassium dichromate solution was fed in a small amount of the potassium dichromate embryonating medium, to chicks No. 2 and No. 3. Salt flotations made at frequent intervals following the administration of the culture were negative for capillaria worm eggs. Post-mortem examination of the chicks 55 days after the eggs were given showed that they had not become infected.

Experiment 4. Up to this time the eggs administered to experimental chicks were given in a single dose. Since it was possible that repeated exposure of a chick to embryonated eggs might effect the transmission of C. caudinflata, the chick in this experiment received smaller amounts of culture over a longer period of time. This culture had been in 2.5% potassium dichromate solution for 25 days when the first dose was given. The droppings of 2 chicks, No. 150 and No. 151 had been examined at least once per week from the time of hatching in order to assure their freedom from capillaria worm infection. On July 1, 2 drops of culture containing approximately 100 eggs per drop were given in a gelatin capsule to chick No. 150. In the same manner the following doses were given: July 3, 4 drops; July 4, 3 drops; July 6, 4 drops; July 14, 16 drops; (motile embryos observed on this date) July 20, 10 drops; July 24, 10 drops; and on August 3, 10 drops from 4 combined cultures, the oldest one having been collected 235 days previously. Chick No. 151 was not given any capillaria worm eggs and was held in the cage as a control. No capillaria worms were found in chick No. 150 when posted 20 days after the last eggs were given. Chick No. 151 was likewise negative for capillaria worms when killed.

Experiment 5. There was a possibility that subjecting embryonated eggs of C. caudinflata to low temperature for a

period of time might in some way cause them to become infective to chickens. Two cultures of eggs which had been collected 45 days and 74 days previously were freed of the potassium dichromate embryonating medium and were placed in tap water in the bottom of a refrigerator where the temperature was held at approximately 6°C. After the culture had been in the refrigerator 75 days, approximately 100 eggs were given to each of 2 chicks, 2 weeks old. A similar number of eggs was given to each chick on 12 different days throughout the following month. Post-mortem examination of these chicks 6 weeks after the last eggs were given failed to reveal the presence of any capillaria worms. One of the chicks, however, was host to a single specimen of Ascaridia lineata, a few eggs of this species having been present in the capillaria egg culture.

Experiment 6. All previous attempts at direct transmission having met with failure, the writer decided to give a large dose of embryonated eggs to a chick, collect the eggs from the droppings passed during the following 24 hours and feed them to a chick again. It was suggested that the first passage might in some way alter the eggs so that they would hatch and develop when fed again.

A 52-day old culture of C. caudinflata eggs was freed of the 2.5% potassium dichromate embryonating medium and was fed immediately to battery raised New Hampshire chick No. 3771 approximately 4 weeks old. The following day the eggs were

collected by centrifugation and salt-flotation and were again fed to the same chick. Motile embryos were observed at this time. The same procedure was followed on the next day but this time the eggs collected from the droppings passed during 24 hours were fed to a New Hampshire chick No. 3772, 4 weeks old. The droppings from this chick were accidentally discarded by an attendant thus preventing further passage of the eggs through the chick. Chick No. 3771 died from an undetermined cause on the 20th day after the first eggs were given. No capillaria worms were found at post-mortem examination. Chick No. 3772 was killed 9 weeks after it received the capillaria eggs and it was also free of capillarids.

Experiment 7. Although repeated passage of embryonated eggs through a chick did not render them infective, there was still the possibility that continued exposure to digestive juices of the fowl might cause the eggs to hatch *in vitro* or when subsequently fed to chicks they might be infective. Gizzard and duodenal contents were collected separately from the digestive tracts of several chickens and the liquid was removed from the solid food substance by means of a suction filter. Five cubic centimeter portions of the 2 types of fluid were placed in separate test tubes and equal parts of each were mixed in a third tube. Then, eggs of *C. caudinflata* from a combined culture, part of it having been collected 75 days previously and held in 2.5%

potassium dichromate solution, the other part 57 days old and embryonated in tap water, were placed in each tube and the tubes were incubated at 35°C. The eggs were examined at frequent intervals for signs of hatching but none was observed. After 10 days incubation the eggs from all 3 tubes were given to one White Wyandotte chick No. 3775. Salt flotation examination of the droppings of this chick made from time to time did not disclose the presence of any capillaria worm eggs. Post-mortem examination of the chick 50 days after the eggs were given showed that it had not become infected.

Experiment 8. Since it was possible that embryonated eggs of C. caudinflata might be rendered non-infective by the potassium dichromate solution, eggs of this species were collected and embryonated in tap water. When the culture was 35 days old it was found to have an abundance of eggs containing motile embryos. Twelve 1 cc. doses of the culture were administered to a 15-day old chick No. 236, over a period of 30 days. This chick died of a tracheal infection 34 days following the initial dose of capillaria worm eggs. By post-mortem examination it was found to be uninfected.

Experiment 9. Only one attempt was made to infect a chick with adult worms of this species. Approximately 400 adult worms identified as C. caudinflata were collected from a chicken received from Missouri. These worms were immediately pipetted into the crop of a New Hampshire chick 6-weeks old.

Post-mortem examination of the chick 45 days later showed that the worms had not become established in its intestine.

Experiment 10. Hundreds of eggs of C. caudinflata were placed in a glass jar containing moist soil in an attempt to infect some earthworms with this capillarid. After the eggs had been in the soil several days, salt flotation examination revealed larvae which had some resemblance to the larval forms of C. columbae illustrated by Wehr (1939). A few of these larvae were given to chick No. 130 but post-mortem examination of this bird 52 days later showed that it had not become infected. Since there was the possibility that the larvae observed were free-living nematodes which had been brought in with the soil, some soil was heated for 1 hour at a temperature of 100°C. in order to kill any nematodes which might be present. The soil was then placed in a 2 oz. glass jar and moistened with tap water. A 6-weeks old culture of embryonated eggs of C. caudinflata was washed free of the potassium dichromate embryonating medium and was then sprinkled on the surface of the soil. Water was frequently added to the jar in order to keep the soil moist.

Samples of the soil examined from time to time by the salt flotation technique revealed a few non-motile larvae but the writer could not be sure of their identity. However, one notable fact was the absence of capillaria worm eggs. Since experience has shown that both embryonated and unembryonated eggs

of C. caudinflata readily come to the surface of a saturated salt solution no explanation was available at the time for the failure to recover the eggs.

The soil in the jar was given in 5 separate portions over a period of 10 days to chick No. 3768, the first portion being given 32 days after the eggs were placed in the soil. New Hampshire chick No. 3769 was given no soil and was held in the same cage as a control. Post-mortem examination of chick No. 3768, 41 days after receiving the first portion of the soil, showed that it was uninfected with capillaria worms. The control was likewise uninfected when it was examined 17 days later.

Experiment 11. A second experiment was conducted in order to determine whether contact with moist soil might in some way cause the eggs of C. caudinflata to become infective. Motile embryos were observed in a 29-day old culture which had been embryonated in 2.5% potassium dichromate solution. The culture was washed free of the embryonating medium and was then transferred to a petri dish containing soil which had been heated for 1 hour at 100°C. Five weeks later the soil was fed to a 4-weeks old chick, No. 3770. No capillaria worms were found in this chick when it was posted 38 days following the administration of the soil.

Experiment 12. After chickens known to be infected with C. caudinflata had been brought to the research farm in

Charles City, it was repeatedly observed that a few chickens hatched and raised to maturity on these premises became infected with the same species of capillaria. In one recorded case, a mature female worm identified as C. caudinflata was recovered from a 140-day old Black Minorca cockerel No. 795, purchased from a commercial hatchery as a day-old chick.

The following experiment is an effort to transmit C. caudinflata to chicks by confining them with an infected bird in a pen where the droppings were allowed to accumulate in an attempt to provide conditions favorable for direct transmission. A small pen 4 feet long and 3 feet wide was constructed in a brooder house where the temperature was maintained at 70° to 100° F., during the cold months of the year, by means of an oil-burning brooder stove. A 3-inch layer of soil was placed in the bottom of the pen. This soil was previously sterilized in an autoclave for approximately 1 hour at 15 pounds pressure. Since spore-forming bacteria placed in tubes in the center of the cans of soil were killed, it is believed that no nematode larvae or other animal life which might serve as an intermediate host to the capillarids could have survived.

A White Rock hen passing eggs of C. caudinflata was placed in the pen with 1 New Hampshire chick and 1 White Wyandotte chick 2 days old. Two months later the droppings

of the hen no longer contained capillaria worm eggs so she was replaced by an infected Buff Orpington rooster. At the same time, 2 more battery raised New Hampshire chicks 8-weeks old were also placed in the pen. Cultures of embryonated C. caudinflata eggs were added to the soil from time to time and the droppings of the chicks were frequently examined by salt flotation.

One chick which had been in the pen for 83 days died. No capillaria worms could be found in this bird but 3 Ascaridia lineata were recovered from the intestine showing that conditions in the pen were favorable for the transmission of at least one species of nematode. Post-mortem examination of the other 3 chicks, 3 months later showed that none of them had become infected with C. caudinflata.

Experiment 13. A 38-day old culture of C. caudinflata eggs, embryonated in tap water, at room temperature, was allowed to dry up over night. On the following morning, water was added and a few of the eggs were placed on a microscope slide for observation. Within 20 minutes many of the eggs hatched. The larvae became very active in the eggs and finally their bodies were thrust out through the operoular openings. After the larvae had left the eggs they remained actively motile for several hours, thrashing about in a manner resembling the motion of free-living nematodes. Examination of the remainder of the culture under a binocular

microscope showed that a high percentage of the eggs had hatched. The entire culture was fed to 4 chicks, two 3-weeks old Minorcas No. 1 and No. 2 and two 2-weeks old Barred Rocks No. 3 and No. 4. Chicks No. 1 and No. 3 were posted 34 days later and chicks No. 2 and No. 4 were posted 95 days later. No capillaria worms were found.

Experiment 14. A 43-day old culture of C. caudinflata eggs, embryonated in tap water at room temperature, was filtered in order to hasten the drying of the eggs. The filter paper was completely dry 3 hours after the filtration. A few eggs were scraped from the filter paper the next morning and were placed in a drop of water on a slide. Larvae hatched from these eggs in about 30 minutes. Twenty-two hours after the culture was filtered, the paper with the eggs adhering to it was cut up and fed to two 9-day old Minorca chicks, No. 5 and No. 6. A third chick received no eggs and was held as a control. These chicks were killed 32-34 days later but none of them had become infected with C. caudinflata.

b. Experiments on the transmission of C. caudinflata by various intermediate hosts

(1) Grasshoppers

Experiment 1. Two laboratory raised grasshoppers identified as Melanoplus differentialis[1] were given embryonated

[1] Identification checked by Dr. C. J. Drake, State Entomologist, Ames, Iowa.

eggs of C. caudinflata. One grasshopper died 2 weeks after receiving the eggs. The other one was killed 2 weeks later but no worms were found in either of these grasshoppers. Examination of fecal pellets from the grasshoppers following the administration of the culture showed that the eggs were capable of passage through the digestive tract of the insects without hatching and since the embryos retained their motility the eggs were apparently unharmed. Since worms were not found in the grasshoppers, no attempt was made to infect a chick.

Experiment 2. During the months of July and August, 1939, more than 100 grasshoppers representing at least 3 species (chiefly Melanoplus femur-rubrum and a species of green meadow grasshopper--Tetigoniidae) were collected from a chicken pen where several birds infested with C. caudinflata were kept. These insects were given to chick No. 87 but post-mortem examination of the bird 4 weeks later showed that it had not become infected. A second chick, No. 137, 4-weeks old also received 10 grasshoppers from the same pen. When the chick was killed 87 days later no capillaria could be found.

Experiment 3. Embryonated eggs of C. caudinflata were given on blades of grass to 4 Melanoplus differentialis and one M. femur-rubrum on August 5, 1939. Two weeks later one M. differentialis was fed to chick No. 118. The chick

received another M. differentialis 8 days later and the other 3 grasshoppers were fed the following day. The chick was killed 26 days after it received the last grasshoppers but no capillaria could be found.

Experiment 4. Embryonated eggs of C. caudinflata were added to a mixture of bran, orange juice and malt and the moistened food was given to 4 Melanoplus differentialis and 6 M. femur-rubrum on January 24, 1940. The grasshoppers consumed the food readily and are known to have eaten the capillaria eggs since some of them were found in the fecal pellets. The eggs used in this experiment were from 3 potassium dichromate cultures, 118 days, 107 days and 72 days old respectively, combined with a 41-day old culture embryonated in tap water. Eggs of this culture were given to the grasshoppers practically every day for approximately 2 weeks. One M. differentialis was fed to a Black Minorca chick No. 3776, 2 weeks after the first eggs were given. The 3 remaining grasshoppers of this species were fed to the same chick 3 weeks later. When the chick was examined 30 days after it had received the last grasshoppers, no capillaria worms could be found. A New Hampshire chick 4-weeks old, No. 3791 received one of the above M. femur-rubrum 20 days after the first capillaria worm eggs were given. A second chick, White Wyandotte No. 3774, received one grasshopper of the same species 30 days after the culture was first

given and again received 2 more M. femur-rubrum 4 days later. Post-mortem examination of these 2 chicks one month later showed that neither of them had become infected with capillaria worms.

(2) Beetles

Four species of beetles have been used in attempts to transmit Capillaria caudinflata to chickens, namely, Tenebrio molitor, Tenebroides mauretanicus, Tribolium confusum and Aphodius sp.

Experiment 1. Embryonated eggs of C. caudinflata were given on several different occasions to some beetles identified as Tenebrio molitor.[1] Since the beetles had not been watered for some time they drank the culture readily. Approximately 7 weeks later, 11 of these beetles were fed during a period of 12 days to a 4-weeks old chick, No. 159. Twenty-five days after the last beetles were given, the chick was killed and examined for capillaria worms but none were found.

Experiment 2. Embryonated eggs of C. caudinflata were placed in an 8-ounce glass jar containing adult and larval beetles of the species, Tenebroides mauretanicus[1] and larvae of Tenebrio molitor. Eggs were added to the culture on 3 subsequent occasions. Forty-five days after the culture had been given to the beetles, chick No. 180 was given 2 larval T. mauretanicus, 2 adults of the same species and

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[1]Identification checked by Dr. C. J. Drake, State Entomologist, Ames, Iowa

8 larval T. molitor. Four days later 3 more adult cadelles (T. mauretanicus) were given and on the following day the chick received 30 T. molitor larvae. Post-mortem examination of this chick 3 months later showed that it had not become infected with capillaria worms.

Experiment 3. Several laboratory raised T. molitor beetles were given embryonated eggs of C. caudinflata from a 38-day old tap water culture. The beetles drank from the culture very readily. Culture material was offered to the beetles daily for a week. A White Leghorn chick, No. 3932 was given 12 of these beetles 23 days after they received the capillaria worm eggs. Chick No. 3790 received no beetles and was kept in the same cage to serve as a control. Post-mortem of these chicks 3 months later showed that they had not become infected with capillaria worms.

Experiment 4. One chick, No. 88, was given one cadelle beetle (T. mauretanicus) which 8 days previously, had been offered embryonated eggs of C. caudinflata from a 48-day old culture. Twelve days later the chick was given 2 cadelles from the same lot of beetles. Post-mortem examination of the chick was made 33 days later but no capillaria worms were found.

Experiment 5. Chick No. 149 received approximately 140 Tribolium confusum which, 6 days previously, had been offered embryonated eggs of C. caudinflata from a 44-day old

culture. This chick received about 50 more beetles from the same lot 5 days later. When post-mortem examination of the chick was made 33 days later no capillaria worms were found.

Experiment 6. Embryonated eggs of C. caudinflata were placed in a jar containing approximately 100 confused flour beetles, Tribolium confusum. Eggs were again offered on several subsequent occasions. Twenty-five of the beetles were fed to chick No. 108, 49 days after they received the first capillaria worm eggs. When the chick was killed 75 days later no capillaria worms could be found./

Experiment 7. Approximately 40 dung beetles, Aphodius sp. were collected from a manure pile where the droppings of chickens infected with C. caudinflata had been allowed to accumulate. These beetles were fed to chick, No. 142, 3 weeks old. The same chick was given 6 more dung beetles from a jar to which embryonated eggs of C. caudinflata had been added 31 days previously. Twenty-five more dung beetles from the same jar were given to the chick 2 days later. Post-mortem examination of the chick 38 days after it received the last of the beetles showed that it had not become infected.

(3) Other Arthropods

In addition to the experiments just recorded, several attempts were made to use other arthropods as intermediate hosts for the transmission of Capillaria caudinflata to

chickens. In one instance a large number of house fly larvae (Musca domestica) were collected from a chicken manure pile and were confined in a jar containing hundreds of C. caudinflata eggs until adults emerged. One attempt to infect a chick by giving it about 140 of the adult flies was unsuccessful. In another instance, approximately 60 small reddish-brown ants of undetermined species were collected from around droppings of chickens infested with C. caudinflata. These ants were given to a young chick but transmission of capillaria worms in this manner was not accomplished. An attempt was also made, without success, to use sow bugs, (species undetermined) as intermediate hosts to C. caudinflata.

(4) Natural transmission experiment

Experiment 1. All attempts to infect chickens with Capillaria caudinflata by direct means having met with failure, the writer investigated various organisms commonly found in poultry yards in order to determine their possible role as transmitting agents. During the summer of 1939 and the spring of 1940, earthworms of the species Lumbricus terrestris and another smaller species, Helodrilus sp., were used in several experiments in an attempt to transmit this capillarid but for some undetermined reason the chicks did not become infected. On this account, the writer turned to other possible transmitting agents such as grasshoppers, beetles, house fly

larvae and sow bugs. Since no success was obtained with any of these arthropods, 2 outdoor pens approximately 10 feet square were constructed so that chicks could be confined with infected adult birds under more or less natural conditions and thus build up a heavy population of capillaria worm eggs in a small area thereby favoring transmission.

Twenty-four, 54-day old battery raised New Hampshire chicks were used in this experiment. On April 30, 1941, 12 chicks were placed with 2 or 3 adult infected hens in each of the pens. Each of these chicks were checked by salt flotation in order to show that their droppings were free of capillaria worm eggs. During the 25 weeks which these chicks remained in the pens, salt flotation examinations were made at weekly intervals to determine the presence or absence of capillaria worm eggs. On 3 occasions, capillaria worm eggs were observed but subsequent flotations failed to show more eggs, indicating contamination by droppings from the adult hens. One chick, No. 3948 was killed 2 days after a capillaria worm egg was observed in the droppings but no capillarids could be found on post-mortem. Transmission was not accomplished in 5 other chicks which died from various causes during the 25 week period. Each of the remaining test chicks as well as the adult infected birds were sacrificed during the 30 day period immediately following the termination of the fecal examinations. All the test chicks were negative except 2, one of which was host to 1 male and 1 female Capillaria retusa, obtained from the

ceca, the other having 1 female Capillaria caudinflata, in the small intestine. Both species of capillaria worms were obtained by post-mortem from the adult hens used as a source of infection. It is of interest to note that the roundworms Ascaridia lineata, and Heterakis gallinae, and the tapeworms Hymenolepis sp., Rallietina oesticillus and Choanotaenia infundibulum were also found in the test birds. No Rallietina tetragona or R. echinobothrida were found although both these species were present in the adult hens at post-mortem.

(5) The transmission of C. caudinflata to chickens by earthworms

Experiment 1. Having demonstrated the transmission of Capillaria caudinflata to chickens confined with adult infected hens, the writer collected dung beetles, house fly larvae and a large number of earthworms from both pens in order to test their ability to transmit C. caudinflata. Six battery raised New Hampshire chicks 33 days old were used for infection tests as follows: 44 dung beetles (Aphodius sp.) were given to chick No. 383 and 7 fly larvae were given to chick No. 404 on October 23, 1941. Neither of these chicks were parasitized by capillaria worms when they were killed and examined 42 days later. Approximately 100 earthworms from the 2 pens were washed thoroughly to free them of soil clinging to their bodies and were given in 5 lots during the period October 23, 1941 to November 3, 1941

to each of 4 chicks Nos. 364, 380, 401, and 406. Examination of the droppings on November 30, 1941 by the centrifuge salt flotation method showed that all 4 birds had become infected with capillarids. Post-mortem examination showed that 3 of the 4 chicks were hosts to one or more worms identified as C. caudinflata. One male specimen identified as Capillaria retusa was recovered from the cecum of chick No. 401.

Experiment 2. The preceding experiment indicated that earthworms are instrumental in the transmission of C. caudinflata to chickens but did not determine whether they served as true intermediate hosts or as incidental hosts.

On December 4, 1941 about 250 earthworms identified as Helodrilus (Allolobophora) caliginosus[1] were collected from a city lot where no chickens had run for many years. These worms were placed in a wooden box about 8"x8"x4". Three chickens, which had been infected with C. caudinflata by feeding them earthworms collected from one of the pens of the previous experiment were used as a source of culture for this test. Fresh cultures of eggs were poured on the surface of the soil in this box from time to time, in order to build up a moderately heavy population of worm eggs. Since nothing was known about the length of time required for the capillaria worm eggs to become infective to earthworms or about the length of time required in the earthworm, 3 chickens were

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[1] Identification confirmed through the courtesy of Dr. Benjamin Schwartz, Chief, Zoological Division, Bureau of Animal Industry U.S.D.A., Washington, D.C.

given worms from this box at varying intervals of time. A 7-weeks old New Hampshire chick received 5 earthworms from this box, 37 days after the earthworms were first exposed to the capillaria worm eggs. Fifteen days later a second chick of the same breed, No. 940, received 12 earthworms from the box and 12 days later a third chick, No. 969 received 20 earthworms.

Since it is possible that the capillaria worms might develop in the soil, the earthworms serving merely as mechanical carriers of the capillaria larvae, one New Hampshire chick No. 928 was given 10 grams of soil from the box in which the above earthworms were kept. Chicks Nos. 942 and 947 were given no treatment but were kept in similar cages to serve as "cage controls" against the possibility of the chicks acquiring an infection in some unknown manner.

Chick No. 763 died on February 2, 1942, 23 days after receiving the earthworms. Post-mortem examination showed that the bird was infected with 2 male and 5 female capillarids identified as C. caudinflata.

On the 24th and 25th days after chick No. 940 received earthworms, droppings examined by centrifuge salt flotation contained capillaria worm eggs. The same was true of chick No. 969 when it was examined by the same method on the 24th day. Since these birds were needed as a source of egg cultures, they were not killed until about 2 months later. When

chick No. 940 was killed on May 11, no capillaria worms were found. Thus it appears that this chick had lost its infection during these 2 months. Chick No. 969, however, was host to 5 male and 12 female worms identified as C. caudinflata.

Post-mortem examination of control chicks Nos. 928, 942 and 947 failed to reveal the presence of any capillaria worms.

The results of this test further indicated that Capillaria caudinflata was transmitted by earthworms belonging to the species H. (A.) caliginosus.

Experiment 3. Ten earthworms of the species H. (A.) caliginosus collected in the fall and held in a box of soil for about 4 months, were given 1/4 to 1/2 cc. of a 66-day old culture of Capillaria caudinflata eggs. The worm eggs were forced into the digestive tract of the earthworms by means of a capillary pipette inserted into the mouth. In some cases, the digestive tract was completely flushed, forcing the soil out of the intestine. Each drop of the culture contained approximately 15 embryonated C. caudinflata eggs. These earthworms were kept in a small jar of soil until March 4, 1941 when 4 surviving worms were given to an 8-weeks old New Hampshire chick, No. 11.

Two days after the earthworms were given to this chick, 3 chicks of the same breed and age were treated as follows: chick No. 12 was given approximately 750 embryonated C. caudinflata eggs from a 33-35 day old culture; chick No. 13

was given 10 earthworms which had never received any capillaria worm eggs; and chick No. 14 received neither capillaria worm eggs nor earthworms and served as a cage control against direct transmission while the chicks were in the cages. All the chicks were placed in one cage.

When droppings from each of the chicks were examined by the centrifuge salt-flotation method on the 23rd and 24th days (25th and 26th for No. 11) no capillaria worm eggs were found. These chicks were killed during the next 2 days and the digestive tracts thoroughly examined for capillaria worms. One male C. caudinflata was recovered from chick No. 11 on April 9, 1942. All other chicks failed to become infected. Therefore, it appears that the earthworm, H. (A.) caliginosus served as a true intermediate host of the capillarid, C. caudinflata. This test was repeated with a larger number of chicks.

Experiment 4. The foregoing experiments gave evidence that earthworms of the species H. (A.) caliginosus are instrumental in the transmission of the capillarid, C. caudinflata. There are 3 possible ways in which chickens receiving earthworms might acquire the capillaria infection. First, the earthworm might serve as a true intermediate host; second, the earthworm might serve merely as a mechanical carrier of infective larvae developing in the soil where the earthworms were raised; and third, the chicks might acquire the infection independently of the earthworms while confined in

the test cages. Therefore the following experiment was carried out in order to determine which of these methods was correct.

Several hundred earthworms identified as H. (A.) caliginosus were collected in a field more than 1/4 mile from any poultry yard. Hence, it is believed that the soil had no chance for contamination by poultry droppings containing capillaria worm eggs. These earthworms were placed in a clean box of uncontaminated soil and on the following day, freshly collected eggs of C. caudinflata were placed on the surface of the soil. Other cultures were added every second day until 3 cultures containing approximately 12,000 eggs each had been applied.

On April 30, 1942, the 38th day after the first culture of capillaria worm eggs was given, 10 Barred Rock chicks 44 days old were each fed 12 of the above earthworms. Each of 10 chicks from the same hatch received 10 grams of soil from the box of infected earthworms, the soil being shaped into pellets and forced into the crop; a third group of 10 chicks received no treatment other than regular feed and water and thus served as cage controls against any chance infection from unknown sources; and each of the 10 chicks in the fourth group was given approximately 600 embryonated C. caudinflata eggs from a 52-day old culture. In order to test the infectivity of the embryonated eggs in this culture, a large number of them was given to several earthworms.

Administration of the eggs was accomplished by means of a capillary pipette inserted into the oral cavity. Two weeks later, 11 of these infected earthworms were fed to a Barred Rock chick No. 91. When post-mortem examination was made on this chick 24 days later, 1 female C. caudinflata was recovered thus proving the infectivity of the culture.

Centrifuge salt flotation examinations were run on the droppings of chicks receiving earthworms in order to detect the appearance of the capillaria eggs. All 10 chicks were positive for capillaria worm eggs by May 24, 1942. On May 23rd, 24th, 25th and 26th, when these chicks were killed capillaria worms recovered from each of the 10 chicks were identified as C. caudinflata.

When each chick was killed, the intestine was removed and the ceca severed for separate examination. The intestine was slit open, the food material removed and washed through a 100-mesh screen in order to remove fine food particles. The remaining substance was then examined in small portions, using a large pyrex bake dish resting on a black background, and a 2.25 power Zeis binocular head-band magnifier.

The entire lining of the small intestine from the gizzard to the cloacal orifice was scraped from the intestine, washed through the same screen and examined in the manner just described. From these 10 chicks, 275/C. caudinflata worms were recovered, 83 of which were males. One chick had only

7 capillarids while the maximum number found in any chick was 58 worms.

When these post-mortem examinations had been completed, each chick in the 3 control groups was killed and its intestine carefully examined by the above method. No capillaria worms could be found in any of the 30 control chicks.

In this experiment (1) Capillaria caudinflata was transmitted by earthworms of the species H. (A.) caliginosus to Barred Rock chicks; (2) embryonated C. caudinflata eggs, infective to chicks when passed through earthworms, did not infect chicks when fed directly; (3) C. caudinflata was not transmitted to chicks by feeding soil which had been contaminated with thousands of eggs 38 days previously (as shown in other experiments, this was more than a sufficient number of days for the eggs to have become infective to earthworms); and (4) no transmission from unknown sources occurred in any of the cage control chicks. Therefore, it is concluded that earthworms of the species H. (A.) caliginosus served as true intermediate hosts of the intestinal capillarid, Capillaria caudinflata.

Experiment 5. Although the preceding experiment showed that C. caudinflata was transmitted by earthworms to chickens, it did not prove that the earthworms used were artificially infected since no uninfected earthworms from the same source as the infected ones were given to chickens. Therefore, the following experiment was carried out.

About 350 earthworms identified as H. (A.) caliginosus were collected from a heavily wooded lot approximately 1/4 mile from the nearest poultry yard. These worms were divided into 2 equal groups on June 20, 1942 and placed in clean wooden boxes. One group was held as controls while embryonated C. caudinflata eggs were scattered on the surface of the soil in the other box. On July 19, 1942, 12 earthworms from the control box were fed to each of 12, 37-day old battery raised New Hampshire chicks, Nos. 3802-3813 inclusive; 12 earthworms from the infected group were given to chicks Nos. 3814-3825. These chicks were of the same breed and same age as the controls. All 24 chicks were kept in wire floored cages. On August 12, 1942, it was found by salt-flotation examination, that all 12 of the chicks receiving infected earthworms were passing capillaria worm eggs. The droppings of the 12 chicks receiving uninfected worm eggs were negative. On August 12th and 13th, all the control chicks were killed and careful examination of the intestine was made by the method described in the preceding experiment. No capillaria worms could be found. On August 13 when one of the infected chicks, No. 3814 was killed, 7 male and 10 female C. caudinflata were recovered. When the other infected chicks were killed about 3 weeks later the following numbers of C. caudinflata were obtained: No. 3815, 3 females; No. 3816, 4 females; No. 3817, 3 males and 9 females; No. 3818, 5 males and 12 females; No. 3819,

5 males and 9 females; No. 3820, 7 males and 20 females; No. 3821, 16 males and 19 females; No. 3822, 2 males and 11 females; and No. 3823, 10 males and 13 females; No. 3824, 2 males and 4 females; and No. 3825, 6 males and 6 females.

Since all of the chicks receiving infected earthworms became parasitized by capillaria worms whereas none of the chicks receiving uninfected earthworms became infected it may be safely assumed that the earthworms used had no previous infection but were artificially infected in the laboratory. Since the control chicks did not become infected it should be noted that they also served the purpose of cage controls against infection from unknown sources while the chicks were in the cages.

3. The endogenous development of C. caudinflata

a. Length of developmental periods

(1) A method for determining maturity in C. caudinflata embryos

When observations were made on the exogenous development of the eggs of C. caudinflata no definite method was available for determining maturity of the embryo other than general appearance. Thus the embryonation period was presumed to be 11 days. During the investigation, a method of hatching the worm eggs in vitro was developed which was helpful in determining maturity of the embryos. It was previously shown that application of digestive juices of the gizzard and

small intestine of chickens would not stimulate embryonated eggs of C. caudinflata to hatch in vitro. (Exp. 7, p. 51). Although partial desiccation for a period of about 12 hours caused a few of the larvae to emerge when the eggs were returned to water, this method had never proved satisfactory for testing the viability of capillarid eggs because the results were not very consistent. Having demonstrated the transmission of C. caudinflata by means of earthworms of the species H. (A.) caliginosus, it appeared that the digestive juices of these worms might cause the larvae to hatch.

Tap water was forced into the oral cavity of several earthworms by means of a capillary pipette, flushing the entire digestive tract. The contents emerging from the anus were collected and filtered to remove debris. A few drops of the filtrate were placed on a microscope slide with a drop of water containing numerous embryonated C. caudinflata eggs. The larvae in these 29-31 day old eggs showed no motility when examined immediately. After about one minute, however, the larvae began to move and in 2 to 5 minutes most of the eggs hatched. During subsequent trials individual eggs have been observed throughout the entire hatching process. The larva moves sluggishly at first but the movement is quickly accelerated. The anterior end of the larva appears to explore the interior of the egg shell for an opening, finally thrusting its anterior end

out through one of the operoula. The larva then makes its way out of the egg and swims away into the surrounding medium by a rhythmic serpentine motion.

(2) Period of embryonation

This method of hatching provided a valuable tool for the determination of maturity in C. caudinflata eggs, and was used in the experiment which follows.

A C. caudinflata egg culture was collected from droppings passed by several infected chicks during a 5-hour period. Since most of the eggs contained motile embryos by the 10th day, a few drops of the culture were treated with freshly collected earthworm digestive juice. None of the larvae hatched. However, the same sample of earthworm digestive juice produced hatching in a 35-day old culture, thereby proving the activity of the digestive juice sample. On the 11th day, the eggs failed to hatch even after 22 hours exposure whereas eggs from the 36-day old control culture hatched within a few minutes. Although the digestive juice appeared to stimulate the worms to greater motility, the larvae were unable to rupture the egg. Similar treatment on the 12th day caused 3 larvae to hatch. On the 13th day about 25 larvae had hatched within one hour after treatment, thus indicating a much higher percent of maturity than was present on the preceding day.

In this in vitro experiment, C. caudinflata eggs were capable of hatching after a 12-day embryonation period, when

treated with earthworm digestive juice. Thus it is believed that these larvae were completely embryonated and would be infective to earthworms after a 12-day embryonation period.

(3) Developmental period in the earthworm

Experiments have been carried out to determine the time necessary in the earthworm before Capillaria caudinflata becomes infective to chickens. On March 26, a drizzling rain with temperature of 45 to 50°F. caused many earthworms to emerge from their burrows and crawl on the surface of the highway. A large number of these worms were collected and placed in 2 boxes about 8"x8"x4". The following day a 21-day old culture of C. caudinflata eggs was placed on the surface of the soil of box No. 10. The earthworms in box No. 12 received no capillaria worm eggs and were saved as controls against any previous infection of the earthworms.

On the eleventh day following the exposure of the earthworms in box No. 10, to the embryonated capillaria worm eggs, 5 of them were fed to chick No. 29; on the 15th day chick No. 33 received 10 infected earthworms; and on the 21st day chick No. 38 received 10 of these earthworms. On the 15th day chick No. 32 received 20 earthworms from box No. 12 and 6 days later 10 more uninfected earthworms were given. Chick No. 31 received no earthworms thus serving as a cage control.

Capillaria worm eggs appeared in the feces of chick No. 29, twenty-two days after the earthworms were eaten

but were not found in the droppings of chicks Nos. 33 and 38 until the 23rd day. No capillaria worm eggs were recovered from chicks Nos. 31 or 32.

Post-mortem examination of the 5 chicks showed that No. 29 was host to 1 male and 3 female C. caudinflata; No. 33, 2 male and 17 female C. caudinflata and No. 38, 47 male and 81 female C. caudinflata. Capillaria worms were not found in chicks No. 31 or No. 32.

This test shows that Capillaria caudinflata embryos under favorable conditions may become infective to chickens after 11 days in the earthworm H. (A.) caliginosus and that the female capillarids may become mature after 22 days development in the chicken as shown by the appearance of eggs in the feces.

Since the preceding test gave no assurance that 11 days was the shortest developmental period possible in the earthworm, an attempt was made to shorten this period. Earthworms of the species H. (A.) caliginosus, collected from a locality where infection with capillarids was unlikely, were divided into 2 equal groups. The worms in one group were infected with embryonated eggs of C. caudinflata, using a capillary pipette for administration. The other group was held as a control against previous infection.

These earthworms were fed to 3, 4-weeks old New Hampshire chicks at various intervals from the time they had received

the capillarid eggs, as follows: chick No. 478 received 8 worms on the 9th day, chick No. 480 received 8 worms on the 10th day, and chick No. 481 received 8 worms on the 11th day. A fourth chick, No. 479, received 8 earthworms from the uninfected control group on each of these 3 days.

When chick No. 478 was killed on the 26th day after it received the earthworms, one male C. caudinflata was recovered. The control chick No. 479 was examined on the following day but no capillarids were present. Since it was thus shown that C. caudinflata can continue its development in the chicken after a period of only 9 days in the earthworm, it was unnecessary to kill the chicks infected on the 10th and 11th days. These chicks were therefore used for another purpose.

A third experiment was carried out in an attempt to transmit C. caudinflata to chicks, when using periods in the earthworm of less than 9 days. About 350 earthworms of the species H. (A.) caliginosus were collected from a location where worms obtained on a previous occasion were found uninfected with C. caudinflata.

On October 20, 1942 at 5:00 P. M., approximately 250 of these earthworms were infected with C. caudinflata by means of a pipette and were placed in box No. 25. Approximately 100 uninfected earthworms were placed in box No. 27 to serve as a control against previous infection.

On October 21, at 8:00 P. M., 50 of the infected earthworms in Box No. 25 were given to New Hampshire chick No. 499 and 50 of the uninfected earthworms in Box No. 27 were given to New Hampshire chick No. 500. Other New Hampshire chicks Nos. 4775, 4752, and 4757 received 50 earthworms each from Box No. 25 at 5:00 P. M. on October 23, 25, and 27 respectively. On October 29, at 5:00 P. M. New Hampshire chick No. 4753 received 40 earthworms from Box No. 25 and the control chick No. 500 received another 50 earthworms from Box No. 27.

Chick No. 4753 became infected with 1 male and 3 female C. caudinflata worms. None of the chicks receiving earthworms infected with C. caudinflata eggs for periods of 1, 3, 5, and 7 days became infected. The control chick, No. 500, was uninfected. Therefore, in all the experiments conducted 9 days is the shortest developmental period which has produced larvae in the earthworm, infective to chickens.

(4) Developmental period in the chicken

Observations were made on 14 experimentally infected chicks in order to determine the length of time required before the female C. caudinflata worms become mature. Maturity was indicated by the appearance of eggs in the droppings.

Centrifuge salt flotations were run on the droppings of each of the 14 chicks on 1 to 5 consecutive days immediately preceding the appearance of eggs in the droppings. Eggs were found on the 22nd day in 4 of the chicks, on the 23rd day in 8 of them and on the 24th day in 2 of them.

The identity of the capillarids was proven by post-mortem examination of the chicks.

The writer has examined many other chicks for C. caudinflata, on the 24th day after experimental infection. In no case have the eggs failed to appear by that time, in the droppings of chicks which became infected with female worms.

b. Description of developmental stages

(1) The embryo

Hatching, initiated by the application of earthworm digestive juices to embryonated eggs, according to the method described on p. 75 of this paper, produced abundant material for morphological study of the embryos. After hatching, the larvae were usually allowed to remain on the slide until they became quiescent or, they were heated gently over an open flame. It was found that a little acetic acid added to a weak alcoholic solution of iodine was very satisfactory for killing capillarid embryos and often helped to differentiate some structures. This treatment was especially helpful when the larvae were photographed.

The newly hatched first-stage larvae were approximately 175 μ long and 8.6 μ wide (Plate II, Fig. 1 and Plate V, Fig. 1). These larvae, after freeing themselves from the egg shell, moved slowly back and forth. The anterior end was most active, having a halting exploratory motion. This characteristic, carried over to the larvae occurring in the earthworm, was

Plate II.

Larval Stages of *Capillaria caudinflata*

Fig. 1. First stage larva escaping from a *C. caudinflata* egg. x ca 120.

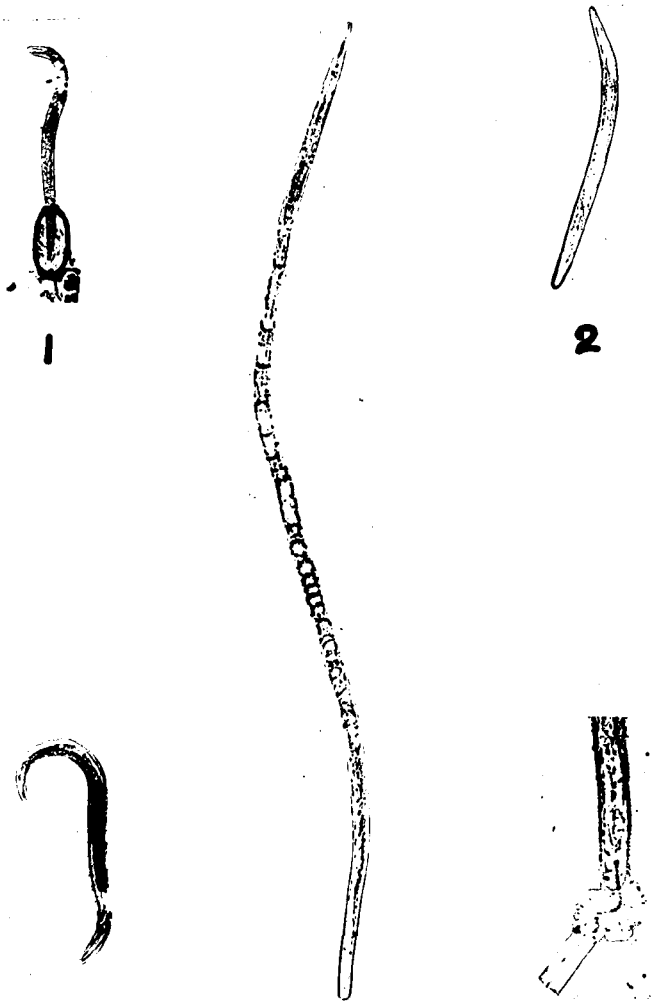
Fig. 2. *C. caudinflata* larva after 5 days development in the chicken. x ca 65.

Fig. 3. *C. caudinflata* larva after 15 days development in the earthworm. x ca 245.

Fig. 4. *C. caudinflata* larva after 11 days development in the chicken. x ca 85.

Fig. 5. Moulting larva photographed on the 12th day of development in the chicken. x ca 120.

PLATE II.



1 2 3 4 5

very helpful in their identification since the action was quite unlike that of rhabditids or other nematode parasites observed in earthworms. In some embryos, a stylet was seen protruding from the anterior end. The posterior end was bilobed. In these larvae, the oesophagus was quite well differentiated but the intestine, like that described for C. columbae (Wehr, 1939) was not well defined. Unlike the

Table 2. Measurements of C. caudinflata Embryos

No. of Worm	1	2	3	4	5	6	7	8	9	10	Mean
Max. Length	182	185	171	175	180	165	164	188	166	174	175.0
Max. Width	8.6	8.6	8.6	9.2	8.0	8.3	8.3	8.6	8.6	9.2	8.6
L. Oesophagus	66	62	59	60	68	61	62	60	68	66	63.2
L. Intestine	116	123	112	115	112	104	102	128	98	108	111.8

Note: All figures represent microns

latter species, however, the cell body did not appear, as a double series of opposing cells, but as a column of irregular shaped cells. The cell body measured about 55 μ in length. Measurements of 10 first stage larvae are shown in Table 2.

(2) Larva found in the earthworm

No moulting forms have been recovered from earthworms. However, due to the considerable growth and development which

occurs in the earthworm and the fact that repeated attempts to infect chickens directly with embryonated eggs have proven unsuccessful, it is believed that the embryo must have moulted at least once.

Since developmental periods of 15 days in the earthworm have consistently produced infective forms, the larvae used for morphological studies were obtained from earthworms which had been infected for 15 days or more. The second-stage larvae were obtained for observation in the following manner. Infected earthworms were placed in a petri dish, submerged

Table 3. Measurements of C. caudinflata Larvae from the Earthworm

No. of Worm	1	2	3	4	5	6	7	8	9	10	Mean
Max. Length	215	180	182	176	160	170	213	185	174	172	182.7
Max. Width	10.3	13.3	10.3	8.9	11.8	11.8	16.2	11.1	13.3	11.8	11.9
L. Oesophagus	81	75	74	64	63	53	80	74	73	64	70.0
L. Intestine	134	105	108	112	97	117	133	111	101	108	112.6

Note: All figures represent microns

in a little water and slit open with a pair of scissors. The internal structures were then scraped from the body wall and the macerated tissues examined under a wide-field binocular microscope, using a magnification of about 100X. The larvae were usually found free of debris, resting on the bottom of

the dish. Rhabditids, on the other hand were generally found entangled in earthworm tissues and other debris, seeming to have a micilaginous substance on the surface of their bodies which is apparently lacking in capillarids.

These capillaria larvae were approximately 183μ long and 11.9μ wide (Plate II, Fig. 3 and Plate V, Fig. 2). The posterior end which bears a membraneous bilobed bursa-like structure was narrowly rounded. No stylet could be observed. The oesophagus was more clearly defined than in the first-stage larva and the larvae had developed a definite intestine which could be clearly seen posterior to the cell-body. The cell-body measured about 61μ. At either end of the latter structure irregular-shaped cells may be seen in the coelomic cavity. Measurements of 10 larvae recovered from the earthworm are given in Table 3.

(3) Larvae found in the chicken

When infected earthworms are eaten by a chicken and the C. caudinflata larvae start to grow, development is confined chiefly to the anterior region for the first few days.

Five C. caudinflata larvae recovered on the 5th day of development in the chicken (Plate II, Fig. 2 and Plate V, Fig. 3) averaged 562μ in length and 22.8μ in width (Table 4). The ratio of the length of the oesophagus to that of the intestinal length was about 3:1. Although no moulting larvae were found at this stage of development, it is probable that

part of them had moulted since 2 of the 5 larvae examined retained the membraneous bilobed bursa-like posterior appendage characteristic of all larvae examined from the earthworm, while this structure was lacking in 3 of the worms. The oesophageal-intestinal junction was clearly visible in 4 of these larvae. The

Table 4. Measurements of Sexually Undifferentiated C. caudinflata Larvae from the Intestine of Chickens

Age of Larvae*	5	5	5	5	5	9	11	11	12	12
Max. Length**	446	432	693	655	538	1.16	1.56	1.49	4.93	3.71
Max. Width***	25.1	22.2	20.7	20.7	25.1	28.10	26.60	28.10	35.40	32.50
L. Oesoph-agus**	315	---	566	525	410	0.90	1.18	---	2.72	2.01
L. Intes-tine**	131	---	127	130	173	0.20	0.38	---	2.21	1.70

*The age given represents the number of days of development in the intestine of the chicken
**Measurements are in millimeters except for those of the 5 day larvae which represent microne
***All measurements of width are in microns

intestine is better differentiated in the 5-day larvae than in those obtained from the earthworm, but there has been only a slight increase in its length. The oesophagus, however, is approximately 8 times as long as it was in the larvae from the earthworm. No bacillary bands were observed.

Measurements of one 7-day larva gave a length of 800 and a width of 28.1μ. The oesophageal-intestinal ratio was not obtained since the junction could not be detected with certainty in this particular specimen. The bilobed posterior membrane observed in some of the 5-day larvae was absent. Thus, the larva resembled the 9-day larva except for its smaller size.

A larva collected on the 9th day, i.e. 9 days after it was eaten by a chicken, had a length of 1.16 mm., the ratio of the length of the oesophagus to that of the intestine being approximately 9:2. The maximum width was 28μ. (Table 4). At this stage of development, the oesophagus appeared as a small tube running through the cell-body (Plate V, Fig. 5). The latter structure, however, was not yet completely organized. The region posterior to the oesophageal-intestinal junction showed only slight development beyond that of the form found in the earthworm. Small refractile bodies present in this area were interpreted as early stages in the development of bacillary bands.

In two 11-day larvae, the mean length was 1.52 mm. and the mean width 27μ. (Table 4). The ratio of the length of the oesophagus to that of the intestine was about 11:4. The principal development over the 9-day larva was an elongation of both the oesophageal and intestinal region, the latter region showing a large number of vacuoles (Plate II, Fig. 4).

Five larvae were obtained from a chicken 12 days after it had received infected earthworms. In 3 of the 5 larvae, the sex could be determined although sexual differentiation was by no means complete. The mean length of 2 female larvae was 5.29 mm. and the width 38.4μ. (Table 5). The ratio of the length of the oesophagus to that of the intestine was about 1:1. No vulva or ovijector was present in these worms. However, a thick-walled vagina, slightly posterior to the

Table 5. Immature C. caudinflata Females

Age of Worm*	12	12	13	17	17	19	19
Max. Length	5.91	4.68	8.30	16.52	15.15	19.71	19.59
Max. Width**	38.40	38.40	40.00	51.70	56.10	70.90	65.00
L. Oesophagus	2.95	2.95	3.64	6.06	6.25	6.50	5.97
L. Intestine	2.96	2.13	4.66	10.46	8.90	13.21	13.62

*The age given is the number of days of development in the intestine of the chicken

**Measurement of width is in microns, all other figures representing millimeters

oesophageal-intestinal junction could be clearly seen. In the anterior region, the oesophagus and the cell-body were well developed, as were 2 gland cells at the junction of the oesophagus and the intestine (Plate V, Fig. 4). The bacillary bands were well formed in these larvae.

One of the larvae, believed to be an immature male, had a length of 3.43 mm. and a width of 31.0μ. (Table 6). This worm was considered to be a male because of the shape of the caudal end (Plate IV, Fig. 2) and the presence of a structure resembling a spicule. The junction of the oesophagus with the intestine could not be observed.

Table 6. Immature C. caudinflata Males

Age of Worms*	12	15	15	17	17	19	19
Max. Length	3.43	8.43	6.61	9.34	9.51	13.09	12.28
Max. Width**	31.00	38.40	39.90	41.40	38.40	48.70	48.00
L. Oesophagus	---	4.14	3.43	4.31	4.30	6.02	5.54
L. Intestine	---	4.29	3.18	5.03	5.21	7.07	6.74

*The age given is the number of days of development in the intestine of the chicken

**Measurement of width is in microns, all other figures representing millimeters

Two sexually undifferentiated 12-day larvae averaged 4.32 mm. in length and 33.9μ in width. (Table 4). The oesophageal-intestinal ratio was about 2.3:1.9. These worms resembled the 11-day larvae in appearance except that the intestine was proportionally longer. One of these larvae was in the process of moulting (Plate II, Fig. 5) but no visible sexual differentiation had occurred at this time.

Growth in the posterior region of the larvae proceeded rapidly at this stage. A young 13-day female (Plate III, Fig. 1) measured 8.30 mm. in length and 40.0μ in width. (Table 5). The ratio of the length of the oesophagus to that of the intestine was about 1:1.3. Little change in appearance over the 12-day female larvae was noted. The reproductive organs were perhaps a little more visible, but like the 12-day female, no genital opening or ovijector was present.

Two immature male worms collected on the 15th day of development in the chicken averaged 7.52 mm. in length and 39.2μ in width. (Table 6). Thus these worms were more than twice as long as the young male recovered on the 12th day of development. The ratio of the oesophageal length to that of the intestine was about 1:1. In these worms, the oesophageal-intestinal junction was clearly differentiated, in contrast to that of the 12-day larva. No definite bursa-like membrane or alae were present but the caudal end was somewhat inflated and it appeared that the 2 processes which later support the bursal membrane of the adult were beginning to make their appearance.

None of the 4 male C. caudinflata worms observed on the 17th day of development in the chicken possessed the bursa-like membrane or the caudal alae characteristic of adult males of this species. The caudal extremity in these worms, as seen in lateral view, showed an enlarged asymmetrical bulb-like

Plate III.

Female *C. caudinflata* Worms

Fig. 1. Photomicrograph showing the appearance of the vulval region in a 12-day *C. caudinflata* female worm. x ca 350.

Figs. 2,3,4,5. Photomicrographs showing variation in appearance of adult *C. caudinflata* female ovijectors.

Fig. 6. Photomicrograph showing the body wall removed and flattened to expose the 2 bacillary bands characteristic of *C. caudinflata*.

PLATE III.

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structure approximately 1.25 to 1.5 times as wide as the body diameter. Two males averaged 9.42 mm. in length and 39.9 μ in width. The oesophageal-intestinal ratio was about 1:1.2.

Ovijectors were present on only 4 of the 8 females observed on the 17th day. However, definite genital openings could be seen on the worms without ovijectors. It would be very difficult to differentiate these immature females from capillarids of the species C. columbae. Therefore, the writer wishes to emphasize the necessity for working with adult worms when attempting specific identification. Maturity can be determined by the presence of eggs in the uterus since it has been determined that the ovijectors are formed before eggs develop. Two females averaged 15.83 mm. in length and 53.9 μ in width. The oesophageal-intestinal ratio was about 1:1.6.

Two 19-day old male C. caudinflata worms were observed and measured. The characteristic bursa-like membrane supported by 2 T-shaped processes was present in both males but caudal alae could be seen in only one of them. Even in this specimen the alae had not yet reached adult proportions. These males averaged 12.68 mm. in length and 48.3 μ in width. The oesophageal-intestinal ratio was about 1:1.2.

The development was practically complete in 7, 19-day old female specimens observed. Partially formed eggs could be seen in the oviducts but they were not yet enclosed in shells. The average length of the 2 females measured was 19.65 mm.

and the average width was 67.9μ. The length of these worms exceeded the average length of 10 female specimens recorded in Table 7./ The oesophageal-intestinal ratio was about 1:2.1.

Table 7. Measurements of C. caudinflata Adult Females

No. of Worm	1	2	3	4	5	6	7	8	9	10	Mean
Max. Length	21.3	19.8	17.5	21.8	18.4	20.1	16.6	15.8	19.4	18.9	18.96
Max. Width*	70.9	69.4	70.9	67.9	68.7	70.9	67.9	60.6	72.4	73.8	69.34
L. Oesoph-agus	6.9	6.5	6.1	6.8	6.6	6.6	6.7	6.2	5.7	6.5	6.47
L. Intes-tine	14.4	13.3	11.4	15.0	11.8	13.5	10.4	10.1	12.9	12.1	12.49
Distance Ant. end to Vulva	7.0	6.6	6.2	6.9	6.7	6.8	6.3	5.8	6.6	6.9	6.58

*Measurement of width is in microns, all other figures represent millimeters

(4) Adults from chickens

Little need be added to the description and figures of adult C. caudinflata worms given by Shipley (1909) and Morgan (1932). The adults are small slender thread-like worms, very difficult to observe until they are removed from the intestine. The anterior portion consists of an oesophagus and cell body while the posterior portion contains the intestine and the genital organs. At the oesophageal-intestinal junction 2 oval or triangular glandular cells may

be seen. The cuticula is faintly striated. A pair of lateral bacillary bands lie just beneath the surface and are very difficult to observe in many specimens. (Plate III, Fig. 6).

The length of the females varied from 15.8 to 21.8 mm. long and were about 70μ wide. The oesophageal-intestinal ratio was about 1:1.9. The females of this species are most easily recognized by the presence of a funnel-like appendage of the vulva which is located just posterior to the cell-body. (Plate III, Figs. 2,3,4,5). This structure which was found in all adult females is a definite structure and not an inflation of the outer wall caused by endosmosis or a prolapsed sheath as suggested by Eberth (1863). Leading to the vulva is a thick walled vagina, a uterus and simple ovary consisting of a single tube. The internal structure is difficult to observe because it is usually masked by the presence of numerous brownish yellow eggs. The posterior end of the female is rounded, with the anus subterminal. Measurements of 10 adult females are recorded in Table 7.

The males of C. caudinflata are generally much smaller than the females. The length of the males varied from 10.3 to 20.3 mm. and were about 58μ wide. The oesophageal-intestinal ratio is about 1:1.2. These males may be distinguished from males of other species found in chickens by the presence of lateral caudal alae and a heart-shaped bursa-like membrane which terminates the posterior end. (Plate IV, Figs. 1,2,3,4,5). Two T-shaped processes support

Plate IV.

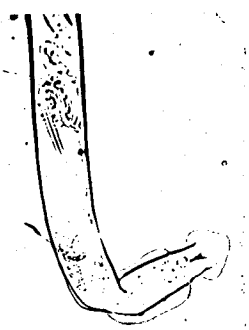
Male C. caudinflata Worms

Fig. 1. Dorsal view of the caudal region of a male C. caudinflata worm collected from the English sparrow. Note the heart-shaped bursa-like membrane and the paired alae.

Fig. 2. Lateral view of the caudal end of a 12-day male C. caudinflata worm collected from the chicken.

Figs. 3,4,5. Lateral views showing the caudal end of adult C. caudinflata worms. Note the T-shaped process supporting the bursa-like membrane. (Fig. 5).

PLATE IV.



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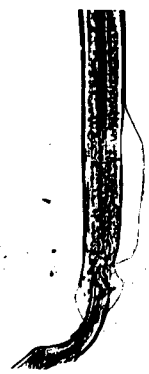


4

5

the latter structure. The spicule is slender, varying from 0.85 to 1.7 mm. in length. The spicular sheath bears no spines but is marked by transverse striation. Measurements of 10 adult males appear in Table 8.

Table 8. Measurements of C. caudinflata Adult Males

No. of Worm	1	2	3	4	5	6	7	8	9	10	Mean
Max. Length	20.3	14.2	15.7	17.4	16.2	11.6	12.2	10.3	10.6	11.2	13.97
Max. Width*	70.9	63.5	69.4	82.5	66.5	44.3	42.9	51.7	54.6	41.4	58.77
L. Oesophagus	8.6	6.7	7.1	7.9	6.9	5.3	4.9	4.5	5.1	5.1	6.21
L. Intestine	11.7	7.5	8.6	9.5	9.3	6.3	7.3	5.8	5.5	6.1	7.76
L. of Spicule	1.7	0.98	1.3	1.4	1.4	0.92	1.0	0.85	0.91	0.99	1.14

*Measurement of width is in microns, all other figures represent millimeters

c. The location of C. caudinflata in the digestive tract of chickens and turkeys

During the course of the various experiments reported, records were kept in order to determine the location of C. caudinflata in the digestive tract. When each chick was killed, the entire digestive tract was removed for examination. No worms were found anterior to the small intestine. The lower digestive tract was divided into 5 portions. The first portion, the duodenal loop, constituted approximately one-fifth

Plate V.

Drawings of *C. caudinflata* Larvae

Fig. 1. Recently hatched first stage larva. Note the bilobed posterior end.

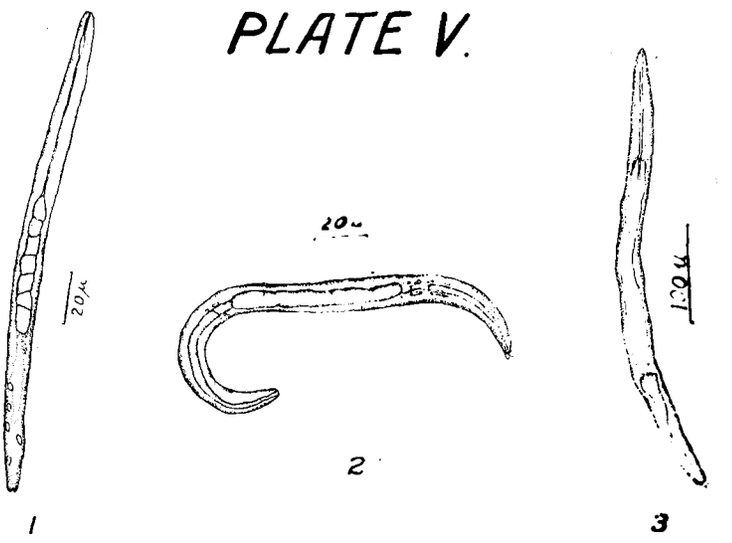
Fig. 2. *C. caudinflata* larva after 15 days in the earthworm. This larva also has a bilobed posterior end.

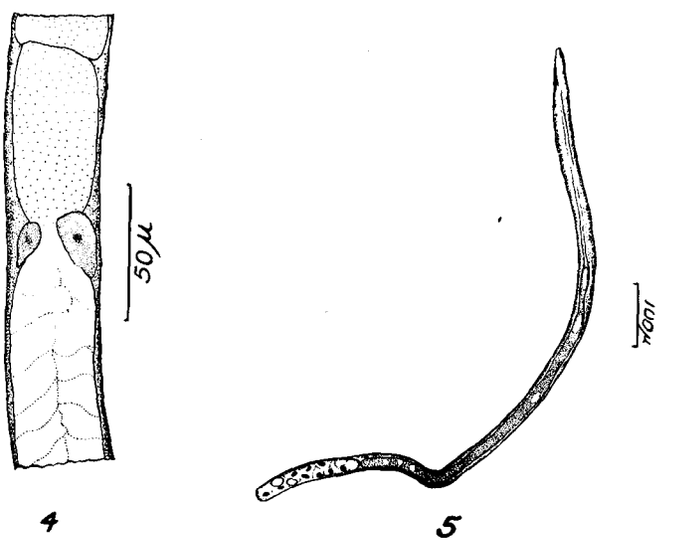
Fig. 3. *C. caudinflata* larva after 5 days in the chicken. Only part of these larvae have a bilobed posterior end.

Fig. 4. Drawing showing oesophageal-intestinal junction of a *C. caudinflata* larva after 12 days development in the chicken. Note the 2 glandular cells found in this part of the body.

Fig. 5. *C. caudinflata* larva after 9 days development in the chicken.

PLATE V.





of the lower digestive tract. The remainder of the lower digestive tract was divided into 4 parts. The duodenum and each of these 4 parts were examined separately for capillaria worms, according to the manner described elsewhere in this paper. The ceca of all birds were likewise examined for capillarids.

Records were kept on 25 chicks and one turkey. Of 551 worms recovered from the chicks, 314 (56.99%) were in the upper one-fifth of the duodenal loop; 182 (33.03%) were in the 2nd fifth; 51 (9.25%) in the 3rd fifth; and 4 (0.73%) in the 4th fifth. No capillarids were found in the last fifth or in the ceca. Of 238 capillarids from the turkey, 126 (52.95%) were in the duodenal loop; 109 (45.79%) in the 2nd fifth and 3 (1.26%) in the 3rd fifth. No capillarids were found in the 4th, or 5th portions or in the ceca. The proportion of male worms found in the turkey was 34.45% whereas the proportion of males in the 25 chicks was 31.95%.

From these data it may be concluded that Capillaria caudinflata is essentially a parasite of the upper half of the small intestine of chickens and turkeys and is particularly abundant in the duodenal loop.

D. Observations Concerning Host-Specificity in C. caudinflata

1. General considerations

The literature regarding Capillaria caudinflata would seem to indicate great freedom so far as host-specificity

in this species is concerned. Due to the inadequate descriptions by earlier workers, it is impossible to draw up an accurate host list, although it appears that this capillarid is able to live in several species of gallinaceous birds.

C. caudinflata was first described by Molin (1858) from the European quail, Perdix coturnix. There is no doubt that Morgan (1932) has collected it from the common fowl, Gallus domesticus although previous records from this host by Rudolphi (1819), Dujardin (1845) and Eberth (1863) cannot be verified from their reports. Mönnig (1933) apparently found this species in chickens of Utrecht. Another host for this species which can scarcely be doubted is the red grouse Lagopus scoticus, reported by Shipley (1909). Clapham (1936) reported finding it in the pheasant Phasianus colchicus. It is also possible that it was seen in the pheasant by Frölich (1802); by Rudolphi (1819); and by Dujardin (1845) although their descriptions leave the determination of their species in doubt. Other hosts which have been reported are the golden pheasant, Chrysolophus pictus; the black grouse or grey-hen, Lyrurus tetrix; the capercaillie, Tetrao urogallus; the partridges Perdix perdix and Perdix cinerea; and the ptarmigan Lagopus lagopus.

Reference has been made by Rudolphi, and other authors to the occurrence of Trichosoma longicollis (=Capillaria caudinflata) in the ceca of Lyrurus tetrix, Tetrao urogallus,

and Perdix perdix. According to the data obtained by the writer concerning the location of C. caudinflata in the digestive tract of chickens and turkeys (see p. 102), it appears that any reference to the presence of capillarids in the ceca of birds would not pertain to C. caudinflata but to some other species, possibly C. retusa.

2. Transmission to turkeys

In order to help clarify our knowledge of host-specificity in C. caudinflata, the writer attempted to transmit this species to several different avian hosts. The first bird other than chickens was the domestic turkey.

Eleven earthworms of the species H. (A.) caliginosus, having been exposed to a 21-day old culture of embryonated C. caudinflata eggs 25 days previously, were fed to a battery raised broad-breasted bronze poult 15 days old, No. 4950 on April 21, 1942. Another poult, No. 4967 received 11 earthworms of the same origin but these earthworms had never been exposed to C. caudinflata worm eggs. A third poult, No. 4955, received no earthworms and was held as a cage control. In order to prove the infectivity of the earthworms, a New Hampshire chick, No. 1755 (46 days old) received 10 earthworms from the infected group. Another chick, No. 1748, received 11 uninfected earthworms and a third chick, No. 31, received no earthworms and thus served as a cage control.

Capillaria worm eggs were present when the droppings of chick No. 1755 were examined by centrifuge flotation 22 days after the earthworms were given. Many capillaria worm eggs were recovered from turkey No. 4952 when it was examined the following day, May 14 but no capillaria worm eggs were found in the controls.

Poult No. 4952 had 82 male and 156 female C. caudinflata worms, recovered by post-mortem on May 16, 1942. Examination of poults Nos. 4955 and 4967 showed that they had no capillaria worms. Chick No. 1755 had 5 male and 8 female C. caudinflata worms while chicks Nos. 1748 and 31 were negative for capillaria worms.

This experiment shows that C. caudinflata may be experimentally transmitted to turkeys by means of earthworms of the species H. (A.) caliginosus. This is the first time that C. caudinflata has been transmitted to turkeys under controlled conditions, using artificially infected earthworms as transmitting agents.

3. Transmission to the English Sparrow

An English sparrow, Passer domesticus, received 10 earthworms of the species H. (A.) caliginosus during a 3-day period. Chicks which received earthworms from this same lot became infected. Since no capillaria worm eggs appeared in the droppings by the 34th day, the sparrow was killed and post-mortem examination was made. Two mature female and one mature male capillarids

recovered from this bird on October 8, 1942 were identified as C. caudinflata. Several immature specimens were also found. The mature females had an ovijector typical of C. caudinflata although no eggs were present in the reproductive organs, thus accounting for the absence of eggs in the droppings. The mature male had the typical heart-shaped bursa-like membrane and caudal alae characteristic of C. caudinflata. (Plate IV, Fig. 1).

A second attempt was made to infect English sparrows with C. caudinflata. The 5 adult sparrows used in this experiment were obtained from a nearby poultry house. Sparrow No. 113 received 13 earthworms of the species H. (A.) caliginosus during a 5-day period starting October 9, 1942. No. 114 received 8 of these earthworms during a 4-day period starting October 10. That these earthworms had been exposed to embryonated capillarid eggs sufficiently long for them to become infective, was proven when 2 battery raised chickens became infected with C. caudinflata after receiving earthworms on October 9 from the same lot of worms given to the sparrows.

When sparrow No. 113 was killed on Nov. 13, 1942, 12 male and 20 female C. caudinflata worms were recovered. Four of the males and 5 of the females were immature since caudal alae and the bursal membrane were lacking in the males and the ovijector was absent in the females. The other males appeared to be fully mature and the females were mature except that no fully formed eggs were found in their uteri.

Salt flotation or centrifuge salt flotation examinations were made daily on the droppings of sparrow No. 114 from Nov. 11 until the bird was killed. Eggs did not appear in the droppings until Nov. 20. Fourteen male and 20 female C. caudinflata worms were obtained from this sparrow when it was killed on Nov. 20. One of the males and 5 of the females were immature. Fully formed eggs were found in only 2 of the adult females.

The results of these tests lead the writer to believe that he has successfully transmitted C. caudinflata to sparrows, using earthworms of the species H. (A.) caliginosus as intermediate hosts. It should be mentioned that the sparrows used in these transmission tests were probably hatched in the vicinity of poultry yards where they spent most of their lives. Thus they may have had opportunity to become naturally infected with capillarids. However, it seems improbable that the birds used in these tests were naturally infected since numerous examinations made on the droppings showed that no capillarid eggs were present. The fact that all 3 controls were uninfected at post-mortem, whereas the 3 sparrows receiving earthworms were hosts to capillarids, is further evidence against natural infection in these sparrows. There would be slight chance that from 3 infected and 3 uninfected sparrows, one would select all 3 of the uninfected birds as controls.

The capillarid specimens found in these sparrows have generally been somewhat smaller than specimens of comparable age

found in chickens, thus indicating that the digestive tract of the sparrow may not provide a completely favorable environment for the development of C. caudinflata. The fact that egg production did not begin until the capillarids had developed 41 days in the sparrow, whereas C. caudinflata worms parasitizing chickens invariably produce eggs on or before the 24th day, further indicates unfavorable environmental conditions. Thus, it seems likely that sparrows are not normal hosts for C. caudinflata.

Conclusions: (1) The English sparrow, Passer domesticus L. is now recorded for the first time as a host of Capillaria caudinflata; (2) evidence has been obtained which indicates transmission of this capillarid to English sparrows by earthworms of the species H. (A.) caliginosus; (3) the digestive tract of the English sparrow does not provide optimum conditions for development of C. caudinflata; and (4) the English sparrow must be considered a potential factor in the spread of the capillarid, C. caudinflata from one poultry yard to another.

The writer attempted to infect 1 White Pekin duck and 1 White King pigeon with C. caudinflata by feeding them infected earthworms of the species H. (A.) caliginosus. Control chicks receiving earthworms from the 2 lots given the duck and the pigeon became infected with C. caudinflata but neither the duck nor the pigeon became infected. It is possible that repetition of these experiments might meet with greater success.

E. Observations on the Longevity of C. caudinflata

The maximum length of time which unhatched embryonated eggs of C. caudinflata may survive, the length of time this species may live in the earthworm, or the maximum age of adults in chickens has not been determined. However, certain observations have been made which indicate that this species is long-lived in the egg stage and possibly in the earthworm, whereas the length of life in the chicken, at least in some cases, is comparatively short.

In one recorded case, eggs of C. caudinflata were collected from infected chicks during the 24-day period from February 4, to February 27, 1941. These eggs were allowed to embryonate at room temperature and were later removed to a refrigerator where they remained for several months at a temperature of approximately 6°C. These eggs were scattered over the surface of a box of soil on December 4, 1941. Fifty earthworms fed to New Hampshire chick No. 1011 on February 9, 1942, produced a heavy infection as shown by the large number of capillarid eggs in the droppings. Fifty earthworms from the same source but which had no access to C. caudinflata eggs were non-infective when fed to a chick. This bird was used as a source of capillaria worm egg cultures until July 7, 1942, about 20 cultures having been collected during this time. Attempts were made to collect cultures on 2 or 3 occasions during the month of August but no eggs were

obtained. When the bird was killed on November 7, 1942, no capillaria worms could be found. It is definitely known that the species of capillarid infesting this chick was C. caudinflata, since the eggs collected from it produced infestation in other chicks when passed through earthworms under controlled conditions.

The history of this bird shows that cultures of C. caudinflata eggs 303-326 days old produced infection in a chick when passed through earthworms. Similar results were obtained with a culture 315-344 days old. It is also shown that heavily infected chickens may lose all their capillarids after a patent period of 5 to 6 months. Although no exact data has been kept on other cases, several chickens confined in small cages have been observed to lose all or most of their infection within a few months.

It has previously been shown that the low temperatures which prevail in Northern Iowa during the winter months are undoubtedly fatal to many C. caudinflata eggs. Never-the-less the test which follows shows that some worms of this species are able to overwinter in this region.

On May 12, 1942, 2 New Hampshire chickens, Nos. 7385 and 7448, about 13 weeks old, were given 50 earthworms from a pen where chickens infected with C. caudinflata had been kept during the previous summer, no chickens having been in the pen since October 22, 1941. On the following day each chick

received 50 earthworms from this pen. Chicks Nos. 7408 and 7444, New Hampshires of the same hatch, were given no earthworms and thus served as cage controls.

Each of the chickens receiving earthworms became infected with C. caudinflata. Five males and 4 females were recovered from No. 7448 and 2 females were obtained from No. 7385. Neither of the control chicks became infected.

This test shows that C. caudinflata survived a period of 202 days from October to May and was then transmitted to chickens the following spring by feeding infected earthworms. It is not known whether this capillarid survived through the winter in the embryonated egg stage, in the earthworm, or in both.

F. Experiments on the Transmission of C. caudinflata by Species of Earthworms Other Than H. (A.) caliginosus

Two attempts were made to transmit C. caudinflata by means of the manure worm Helodrilus (Eisenia) foetidus. On one occasion a chick No. 45 received 2 earthworms of this species after they had been exposed to embryonated eggs of C. caudinflata for 35 days. The chick did not become infected. Seven H. (E.) foetidus, infected by capillary pipette, with embryonated C. caudinflata eggs 26 days previously, were given to chick No. 4473. When this chick was killed 25 days later, no capillarids could be found.

Several attempts to transmit C. caudinflata by using "night-crawlers", Lumbricus terrestris, as intermediate hosts gave negative results.

G. Observations Concerning Capillaria retusa

Although experiments on the life-cycle of C. retusa were not planned for this investigation, certain unanticipated observations are reported at this time. In one experiment (See p. 64), one chick became infected with this species when confined in a pen with adult infected birds. In another experiment (See p. 66), it was further shown that a chick which received earthworms from the above pen became infected. Although it must be admitted that the transmission to these 2 chicks was not adequately controlled, for proving conclusively that earthworms serve as true intermediate hosts to C. retusa, it did show that earthworms may serve as transmitting agents. The following experiment showing transmission through earthworms also substantiate this claim.

In August, 1940, 2 chicks approximately 10 weeks old were obtained from a poultry yard near Bassett, Iowa. Examination of the droppings from these birds showed that they were heavily infected with capillaria worms. Since the owner did not permit post-mortem of the birds, the species was not definitely identified. On March 17, 1941, earthworms were collected from the yard where these 2 chicks had been raised. Six Lumbricus terrestris were given to chick No. 19 while 480 H. (A.) caliginosus

were divided equally between chicks Nos. 17 and 18; chick No. 20 received no earthworms and thus served as a cage control. These chicks were 5 weeks old.

Chick No. 17 died soon after the earthworms were given and consequently was not examined for capillaria worms. On the 27th day after chicks Nos. 18 and 19 received the first earthworms, examination of the droppings by centrifuge flotation showed that they were infected with capillaria worms. The control chick, No. 20, was negative. On post-mortem examination, 2 females and 1 male C. retusa were recovered from the ceca of each of the infected chicks but no capillarids were found in the control chick.

The owner of the poultry yard at Bassett stated that no chickens had been in the yard since November of the previous year. Thus it was shown that capillaria worms of the species, C. retusa are able to overwinter in poultry yards of northern Iowa, either in the unhatched egg stage or in the bodies of earthworms of the species, L. terrestris and H. (A.) caliginosus. It was also shown that C. retusa reaches maturity after a period of 27 days or less, in the body of the chicken.

DISCUSSION OF RESULTS

The reader will note that the review of literature in this thesis is largely a summary of the papers on nomenclature. After consideration of these papers, the writer is convinced that the capillarid dealt with in this thesis is properly called Capillaria caudinflata (Molin, 1858), reasons having been clearly stated in the review of literature for the adoption of this name over several questionable synonyms.

According to the literature, this species was frequently encountered in various gallinaceous birds of the British Isles and the continent of Europe. However, in 1939 the writer reported what is believed to be the first published record of Capillaria caudinflata occurring anywhere in the United States. At that time this species was reported from Iowa, Minnesota, Ohio, Illinois, Wisconsin, Pennsylvania, Missouri, Kansas, Indiana and Michigan. This list is now extended to include Kentucky, New York and West Virginia.

Capillaria columbae has been reported by various investigators from pigeons, chickens or turkeys of Maryland, New Jersey, New York, South Carolina, Pennsylvania and the District of Columbia. The writer (1939) reported C. columbae from chickens of Minnesota, Illinois, Wisconsin, Ohio, Rhode Island and Georgia. The present list also includes Indiana and West Virginia.

Cram (1932) reported Capillaria retusa from Maryland, Washington D. C. and Pennsylvania. The writer (1939) recorded the same species from Ohio and now adds Alabama and Iowa to the list.

The geographical distribution study reported in this paper indicates that C. columbae is the predominant species of capillarid east of the Appalachian mountains whereas C. caudinflata predominates in the mid-west. Only 3 birds infected with C. columbae have been received from localities west of the Mississippi river, all 3 of them from Minnesota.

One wonders that more records of capillarids have not been found from states west of Iowa, Kansas being the only state listed. Since hundreds of chickens have been received from this area, especially from the states east of the Rocky Mountains, it seems likely that capillarids may not be as abundant in this area as they are farther east. The absence of C. caudinflata from arid regions would be understandable, due to the necessity of using earthworms as intermediate hosts, but does not account for their apparent absence in regions having abundant moisture.

Eggs of Capillaria caudinflata, like other closely related nematodes undergo a period of development outside the body of the avian host. It was found that the first cleavage of eggs in tap water cultures held at room temperature usually occurred within 24 hours after the eggs were passed

from the body of the bird. The first indication of motility of the embryo within the egg-shell was observed on the 8th day after the eggs were collected and embryos which appeared to be mature were found after the 11th day. It has been observed that less favorable conditions such as lower or higher temperature and desiccation tend to prolong the incubation period.

Eggs of C. caudinflata can be distinguished from those of C. columbae in several different ways. The difference in the length of the incubation period is one possible method, C. columbae requiring only 6-8 days whereas C. caudinflata requires 11-12 days. The shape of the eggs provide another distinguishing characteristic. Eggs of C. caudinflata are narrower in proportion to their length than those of C. columbae and the latter frequently are more or less asymmetrical whereas the eggs of C. caudinflata are rarely so. Wehr (1939) found that the measurements of 25 eggs of C. columbae vary from 50μ to 55μ in length and from 27μ to 31μ in width. The measurements for a like number of C. caudinflata eggs, as recorded in this paper, were 50μ to 59μ long and 21μ to 24μ wide. A third difference is the character of the egg shells. The egg shells of C. caudinflata are truly punctate shells. When a microscope is focused on the surface of the shell and the light properly adjusted, pinpoint areas of brighter illumination may be seen dotting the surface. One can

see the surface markings even more advantageously under dark-field illumination and especially so in embryonated or partially embryonated eggs. Eggs of C. columbae are also said to have punctate shells but the writer has observed a different type of marking. To describe their surface as having an etched or sculptured appearance conveys a better picture than to speak of them merely as being punctate. As in the case of C. caudinflata, the surface markings appear to have no characteristic design or pattern. Since the eggs of C. retusa have not been studied, it is not known whether they possess any outstanding characteristics sufficiently constant to allow one to distinguish them from eggs of C. columbae or C. caudinflata.

The environmental factors influencing the development of a parasite egg are often of great significance so far as they relate to control measures. The embryonation of C. caudinflata eggs in a 2.5% aqueous solution of potassium dichromate, 1% nitric acid solution and in tap water was readily accomplished. On the other hand, most of the eggs placed in a 1% solution of formalin failed to develop and, with 2% formalin as the embryonating medium, no development occurred at all. Wehr (1939) found that eggs of C. columbae developed in 1% and 2% formalin as well as they did in tap or distilled water, indicating that eggs of the latter species are not as susceptible to the effects of formalin as C. caudinflata. One might suspect that the same would hold true

for other chemicals and that C. caudinflata would therefore be more easily controlled by means of disinfectants. This prediction, however, lacks experimental confirmation.

Embryonated and unembryonated eggs of C. caudinflata subjected to temperatures of approximately 40°C. (104°F.) and -10°C. (14°F.) were adversely effected. In experiments where unembryonated eggs were held at temperatures of 38-42°C. for periods of 23 to 107 days none of the eggs developed to the coiled embryo stage. Two different cultures of embryonated eggs held at 38-42°C. for 42 days, however, showed no indication of degeneration even after remaining at room temperature 8 weeks following treatment.

Unembryonated eggs of this capillarid were capable of developing to the coiled embryo stage when held at 6°C. but did not reach this stage when held at -10°C. for as long as 33 days. However, 58% of the eggs in the latter culture continued to embryonate after removal to room temperature. Unembryonated eggs which were subjected to outdoor winter temperature varying from -21°F. to 68°F. during periods of 44 and 64 days, failed to embryonate. These eggs showed unmistakable signs of degeneration.

Embryonated eggs subjected to a temperature of -10°C. for 6 days were still alive and capable of hatching under the stimulus of earthworm digestive juice, but would not hatch after 14 days of refrigeration. Embryonated eggs subjected

to outdoor winter temperature varying from -21°F. to 68°F. showed no signs of degeneration after 63 days exposure. In the absence of definite viability tests it cannot be definitely stated whether these eggs were dead or alive.

On the basis of these experiments it appears that unembryonated eggs of C. caudinflata withstand low temperatures better than embryonated eggs but the latter are better able to withstand high temperatures. This conclusion regarding the relative effect of low temperature on embryonated and unembryonated eggs coincides with the results obtained by Wehr (1939) who stated that, at the same temperature, embryonated eggs of C. columbae are apparently viable for a shorter time than non-embryonated eggs.

Levine (1937) reported that embryonated eggs of C. columbae were dead 14 days after being dried by an electric fan. Wehr (1939) working with the same species stated that air drying partially embryonated eggs of the same species for 24 hours was lethal to the eggs. On the contrary, the writer has found that drying embryonated eggs of C. caudinflata for 24 hours served in some way as a stimulus for the hatching of the eggs when water was added to the culture.

It should be noted that the temperature extremes to which these capillaria worm eggs were subjected, i.e. approximately 14°F. to 104°F., are within the temperature range of much of the northern half of the United States. It is therefore

probable that many C. caudinflata eggs are actually killed during extreme weather conditions in this region.

The possibility of the direct transmission of C. caudinflata by feeding embryonated eggs to chickens was thoroughly investigated. Carefully controlled experiments were conducted using numerous variations in length of embryonation period, type of embryonating media, age and breed of chicks and method of administration of the eggs, but in no case has the writer been successful in the direct transmission of this species.

All evidence thus indicated that an intermediate host of some type would be necessary for transmission. The writer carried out transmission experiments with various types of invertebrates commonly found in poultry yards. Most of these animals have given negative results, earthworms being the only exception. Those giving negative results were as follows: Grasshoppers--Melanoplus differentialis, M. femur-rubrum and an unidentified grasshopper of the family Tetigoniidae; beetles--Tenebrio molitor, Tenebroides mauretanicus, Tribolium confusuum and Aphodius sp.; flies--larvae and adults of Musca domestica; and undetermined species of ants and sow bugs.

It has been found that C. caudinflata is readily transmitted to chickens through earthworms of the species Helodrilus (Allolobophora) caliginosus. Carefully controlled experiments have shown that this species of earthworm serves as a true intermediate host. Thus there are 3 phases in

the life-cycle of C. caudinflata: the embryonation period of the egg, the developmental period in the earthworm and the developmental period in the chicken.

According to the data recorded in this paper, the entire cycle requires 40-45 days. Eggs treated in vitro with digestive juice of earthworms were able to hatch on the 12th day of embryonation but not before. Thus embryonation requires about 12 days at room temperature, although any substantial deviation from this temperature would certainly alter this period. C. caudinflata larvae have not proven infective to chickens after developmental periods of 1, 3, 5 and 7 days in the earthworm. But 2 chicks have become infected with these capillarids after a period of 9 days in the intermediate host. It is possible that further investigation may show that the required period in the earthworm is less than 9 days since a chick in one experiment became infected with C. caudinflata after receiving earthworms which were placed in a box with unembryonated eggs 18 days previously.

Freshly hatched larvae did not develop when fed to chicks indicating that some time is necessary in the intermediate host before the larvae become infective. Eggs regularly appear in the droppings of chicks on the 22nd, 23rd or 24th day after receiving infected earthworms.

During these experiments, several thousand earthworms have been used. Obviously, it would be almost impossible

to make a specific identification of each individual earthworm. However, specimens have been carefully identified from representative samples taken in the various places from which the worms were collected. Although it is possible that specimens of some other species may have been included in a few cases, the great majority of the worms are known to be H. (A.) caliginosus.

The utilization of earthworm digestive juice for causing C. caudinflata embryos to become motile and to hatch provides a valuable research technique. It was used on one occasion to determine maturity in embryos and once to determine mortality in eggs subjected to low temperature. It is unfortunate that this method was not developed earlier so that other experiments involving the effect of temperature on embryonated eggs could have been repeated.

Studies on the development of C. caudinflata larvae indicated that considerable development occurred in the earthworm, the larvae probably moulting at least once during this period. After the earthworms were eaten by a chicken, the oesophageal region of the larva developed rapidly but there was little growth in the intestinal region for several days. By the 12th day, however, the intestine had grown until it was approximately equal to the length of the oesophageal region. A moulting larva was found on the 12th day in one chicken, together with sexually undifferentiated larvae and

immature male and female worms. The secondary sexual organs such as the caudal alae of the male and the ovijector of the female were not yet present in 15-day specimens. The ovijector did not appear in females until the 17th day of development in the chicken and the secondary sexual organs of the male did not appear until the 19th day.

It was found that C. caudinflata is primarily a parasite of the duodenal loop of both chickens and turkeys. When the intestine was examined in 5 approximately equal parts, it was found that a little over half the worms were in the duodenal loop of the chicken and turkey and that only about 10% of them were found in the lower three-fifths of the chicken intestine, while none were found in the ceca of either the chicken or turkey. Approximately one-third of the worms recovered were males.

Capillaria caudinflata is not host-specific, since this species was transmitted to turkeys and to the English sparrow. Attempts to infect a duck and a pigeon were unsuccessful.

It was shown that C. caudinflata may over-winter in Northern Iowa, surviving a period of about 7 months, and that embryonated eggs may remain viable for at least 10.5 months under laboratory conditions, thus demonstrating considerable longevity in the developmental stages preceeding the actual infection of chickens. However, it was shown that chickens may lose all their infection after a patent period of 5 to 6 months.

The few observations made on C. retusa show that this species is also able to survive the winter in Northern Iowa, that earthworms of the species H. (A.) caliginosus and Lumbricus terrestris may serve as transmitting agents, and that the females reach maturity in the chicken in a period of 27 days or less.

CONCLUSIONS

1. The capillarid of the lower digestive tract of chickens and other avian hosts, formerly referred to as Trichosoma longicolle Rudolphi, 1819 or other questionable synonyms, is properly called Capillaria caudinflata (Molin, 1858).

2. In a geographical distribution study, Capillaria caudinflata was collected from 13 different states, C. columbae from 11 states and C. retusa from 3 states. C. caudinflata was more prevalent than C. columbae in the mid-west but the reverse was true in states east of the Appalachian Mountains.

3. Capillaria caudinflata could not be transmitted by feeding embryonated eggs directly to chickens.

4. Earthworms of the species Helodrilus (Allolobophora) caliginosus served as true intermediate hosts to C. caudinflata.

5. Attempts to transmit C. caudinflata by using earthworms of the species Helodrilus (Eisenia) foetidus and Lumbricus terrestris were unsuccessful.

6. The life-cycle of Capillaria caudinflata required 42-45 days, 11-12 days of which were needed for embryonation of the ova, 9 days for development in the earthworm and 22-24 days for development in the chicken.

7. All attempts to utilize various species of grasshoppers, beetles, houseflies, ants, and sow bugs as intermediate hosts were unsuccessful.

8. Eggs of Capillaria caudinflata can be distinguished from those of Capillaria columbae by differences in their size, shape and the surface markings of their egg shells.

9. Temperatures corresponding to the extreme outdoor conditions in Northern Iowa were detrimental to Capillaria caudinflata eggs. Unembryonated eggs were able to withstand low temperature better than embryonated eggs but the latter were better able to withstand high temperature.

10. Capillaria caudinflata and Capillaria retusa were both able to over-winter in poultry yards of Northern Iowa.

11. When Capillaria caudinflata eggs were treated in vitro with filtered digestive juice of earthworms they hatched within a few minutes thus providing a valuable technique for proving or disproving the viability of the embryos.

12. The various developmental stages in the life-cycle of Capillaria caudinflata have been studied.

13. Capillaria caudinflata was transmitted to a turkey and to 3 English sparrows, but a pigeon and a White Pekin duck failed to become infected after receiving infected earthworms.

14. Development of Capillaria caudinflata was retarded in the sparrow since a developmental period of 41 days was required before eggs appeared in the droppings.

15. Earthworms of the species Helodrilus (A.) caliginosus and Lumbricus terrestris served as transmitters of Capillaria retusa, although it was not determined whether they served as true intermediate hosts or as simple vectors.

16. Capillaria retusa reached maturity in the chicken on or before the 27th day after the earthworms were eaten.

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ACKNOWLEDGMENTS

It is with sincere appreciation that the writer takes this opportunity to thank those who have given assistance during this investigation. Especial mention is due Dr. E. R. Becker whose advice has been particularly helpful in outlining and carrying out the various experiments. The writer gratefully acknowledges permission given by the graduate college and the graduate committee for permitting a large part of this investigation to be conducted in absentia from Iowa State College. Thanks are also extended to Dr. J. E. Salsbury, who has generously provided research facilities for conducting this investigation at Dr. Salsbury's Laboratories, Charles City, Iowa.